

Figure 1: Examples of Nuclease Stable Ribozyme Motifs

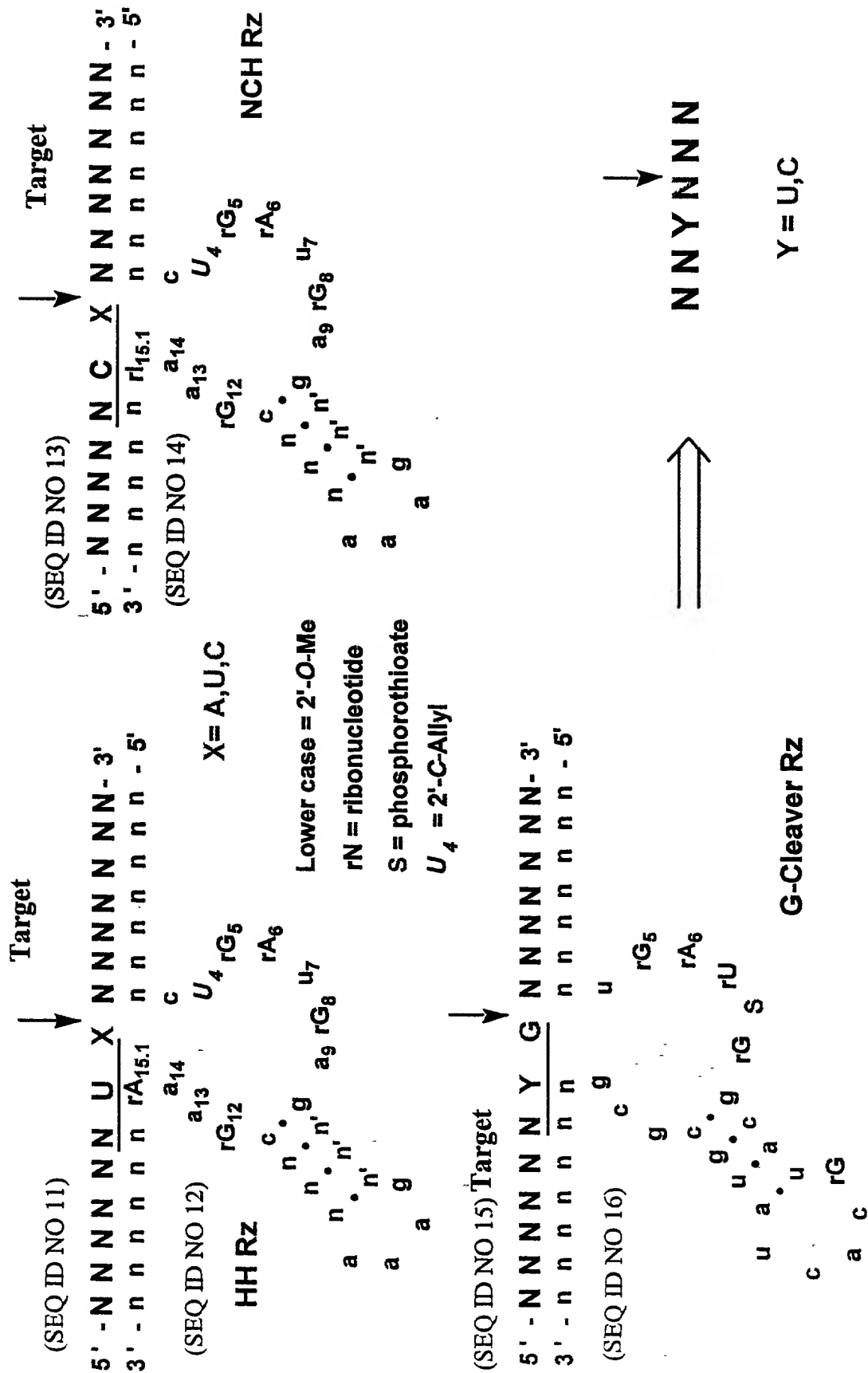
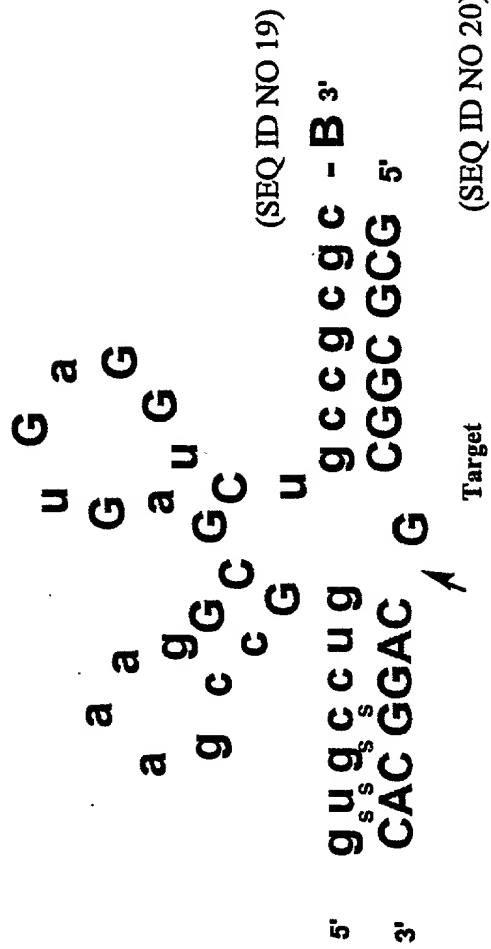




Figure 3: Stabilized Zinzyme Ribozyme Motif

## Zinzyme A-motif RZ

Legend

Uppercase indicates natural ribo residues

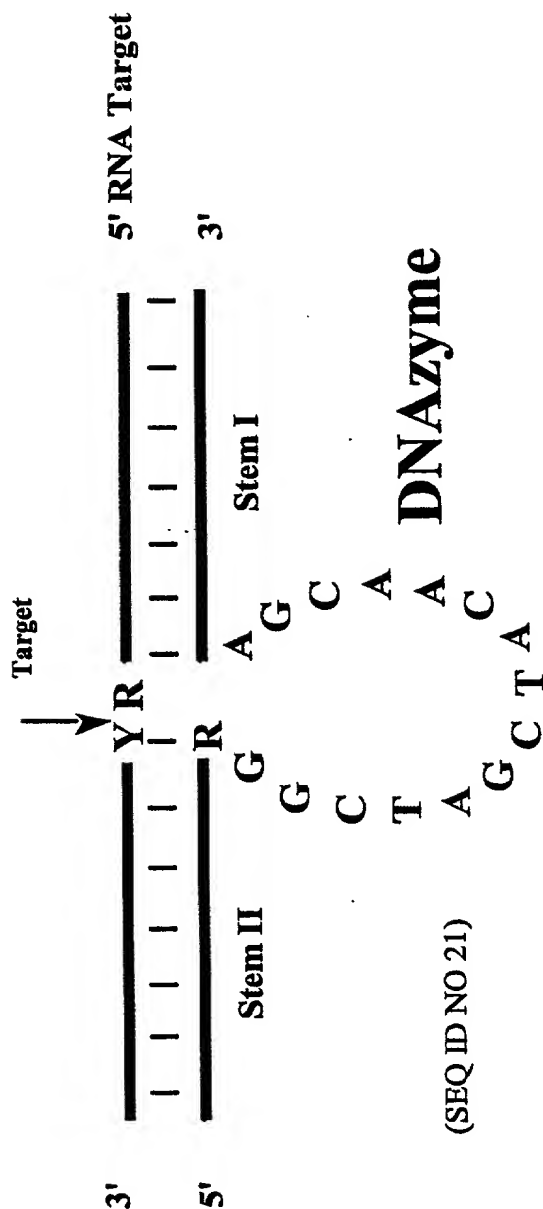
**C** indicates 2' - d-NH<sub>2</sub>-C

Lowercase: 2'-O- Me

Subscript <sub>s</sub> indicates phosphothioate linkage

B: 3'-3' abasic moiety

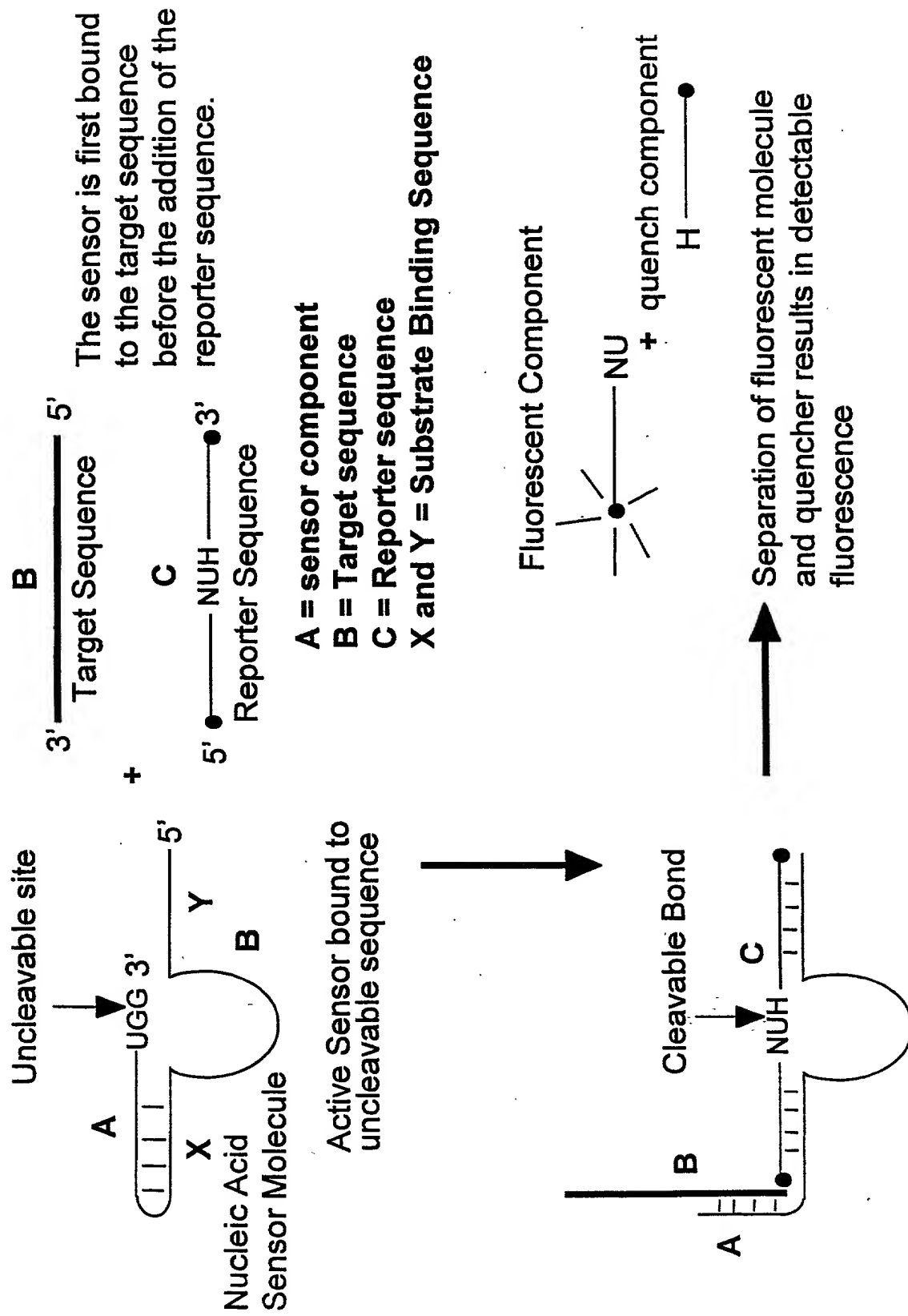
Figure 4: DNAzyme Motif



Legend

Y = U or C  
R = A or G

**Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence**



**Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule**

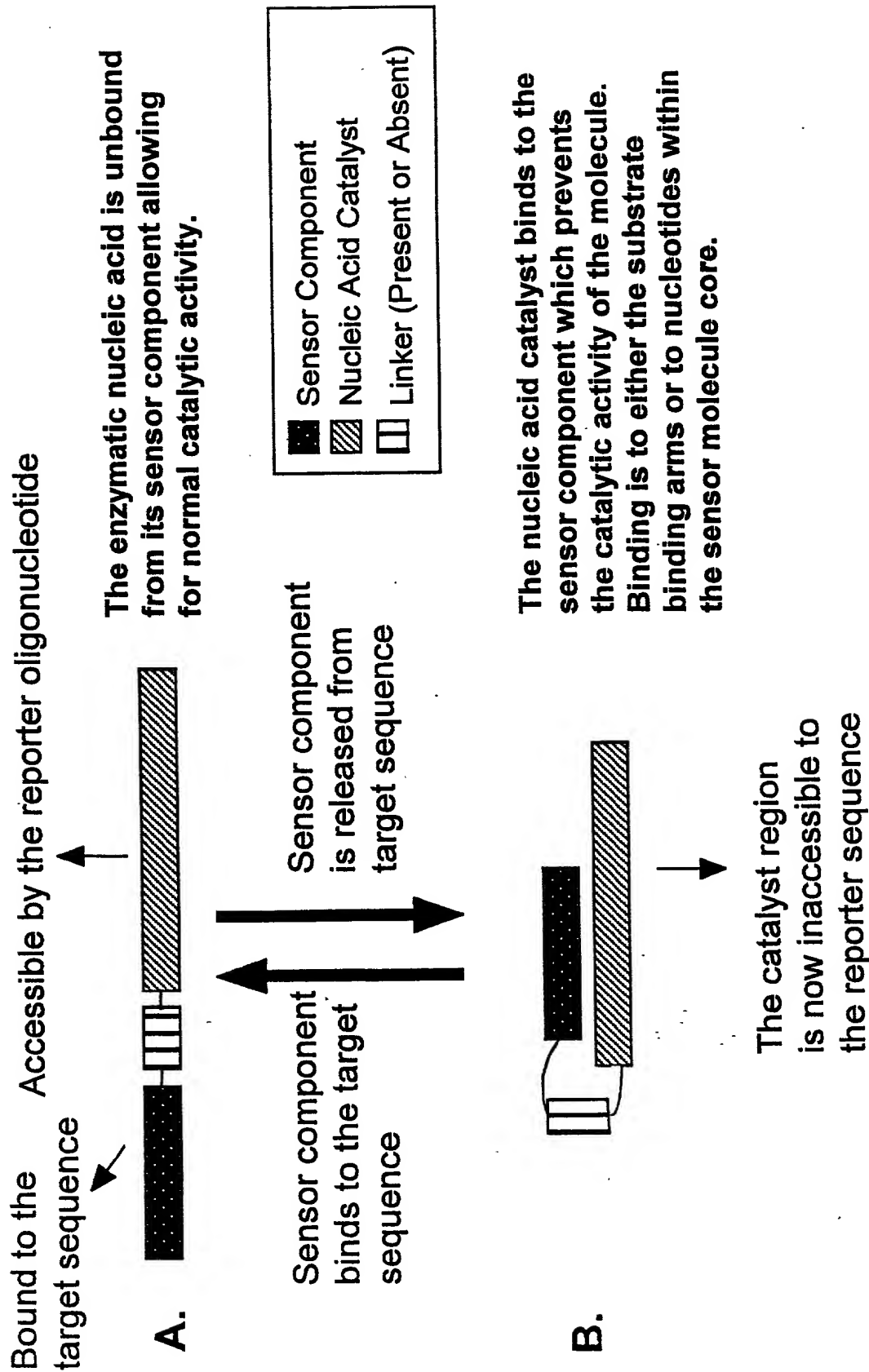


Figure 7. Examples of Nucleic Acid Sensor Molecules

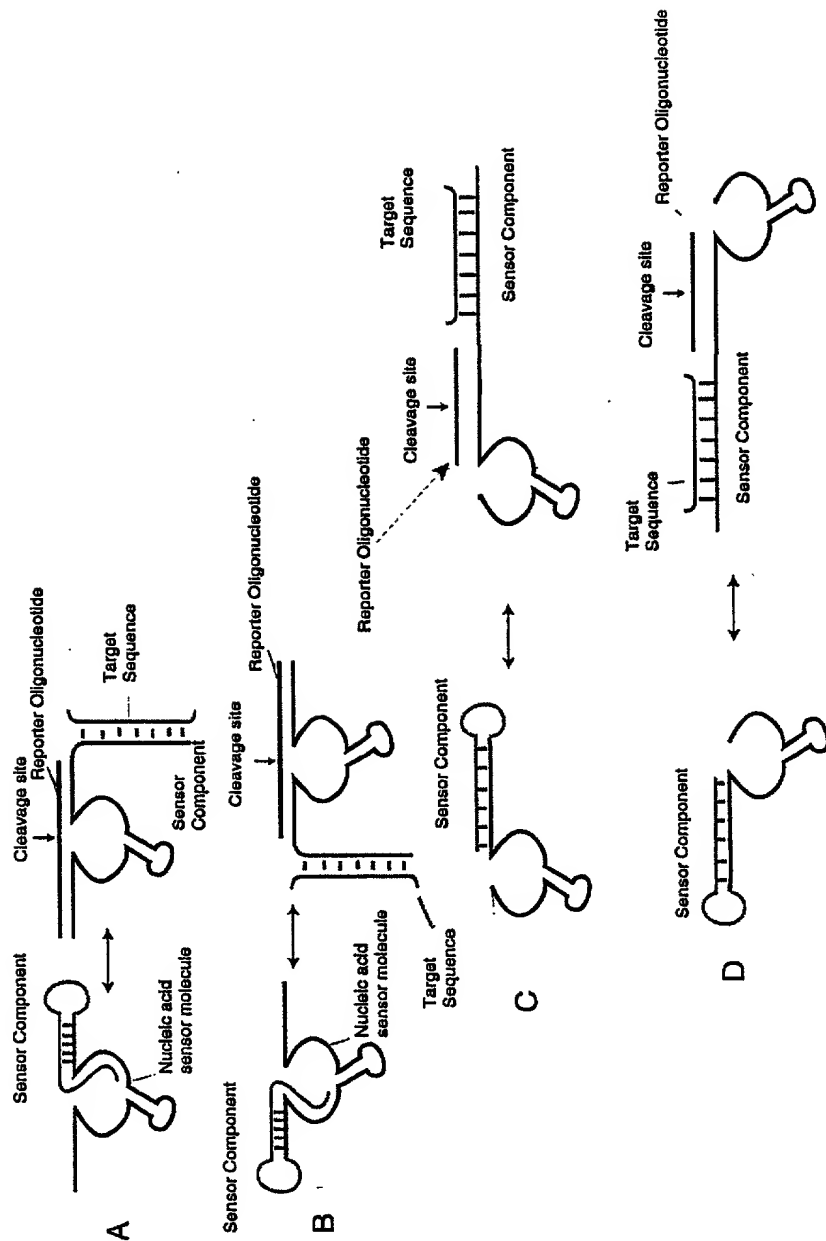


Figure 8. Examples of Nucleic Acid Sensor Molecules

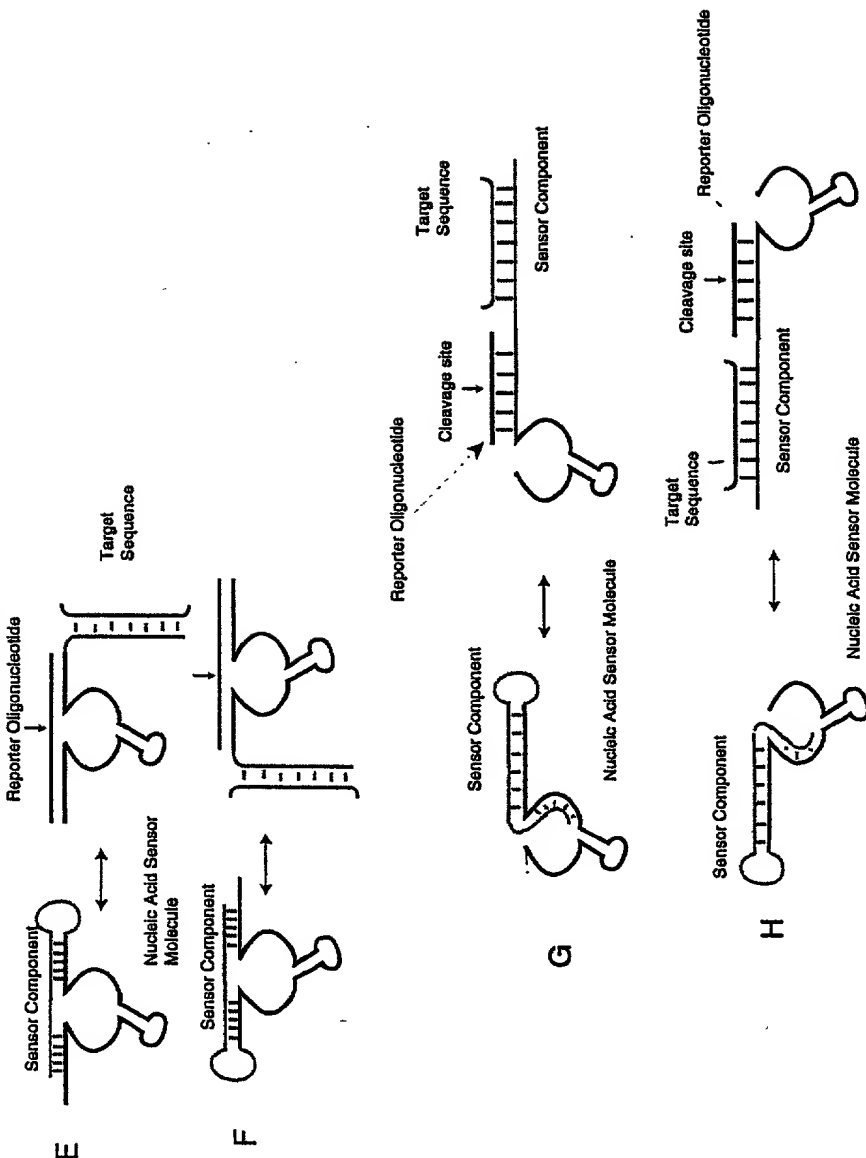




Figure 9. Examples of Nucleic Acid Sensor Molecules

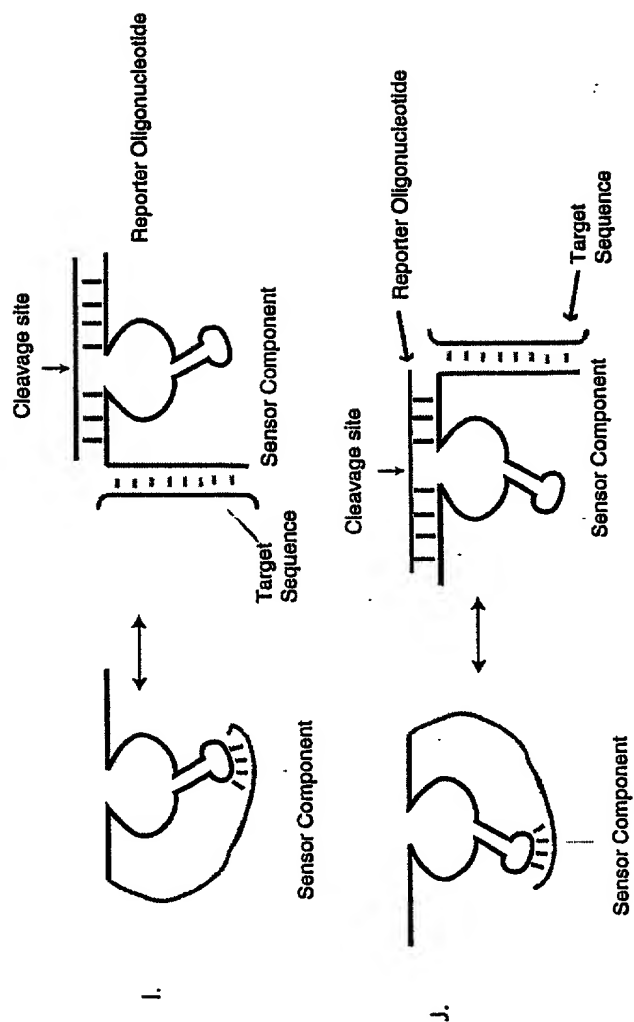
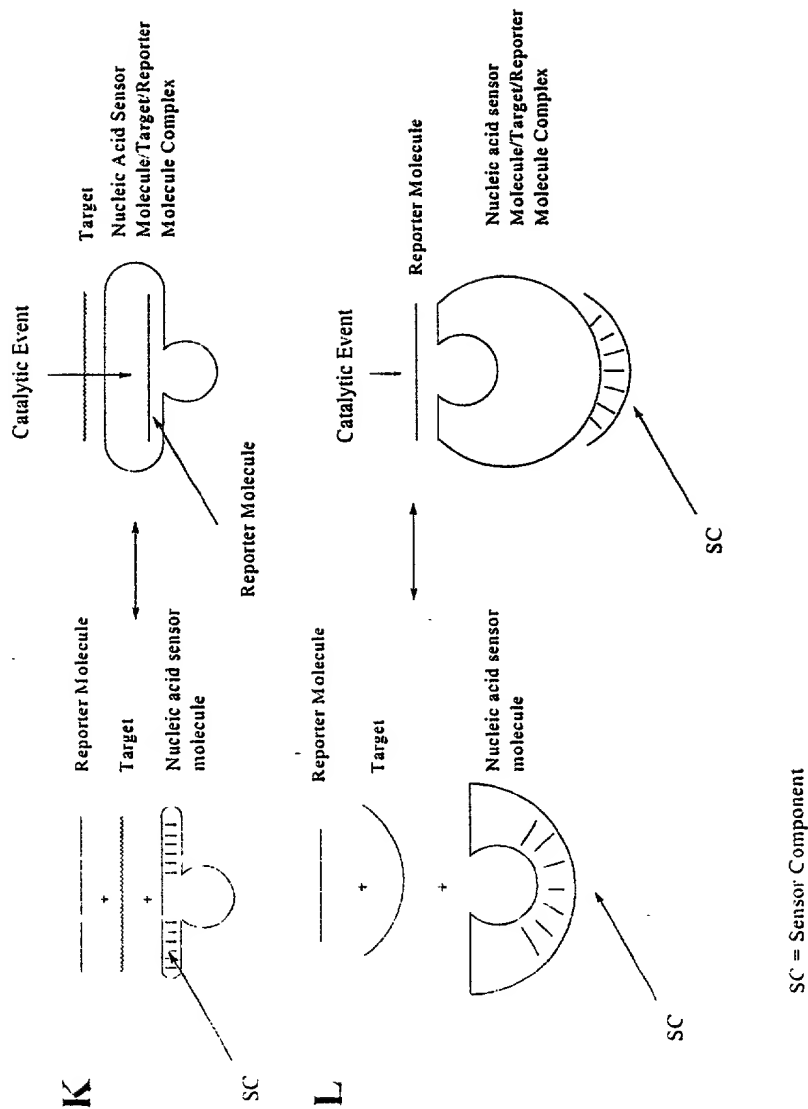
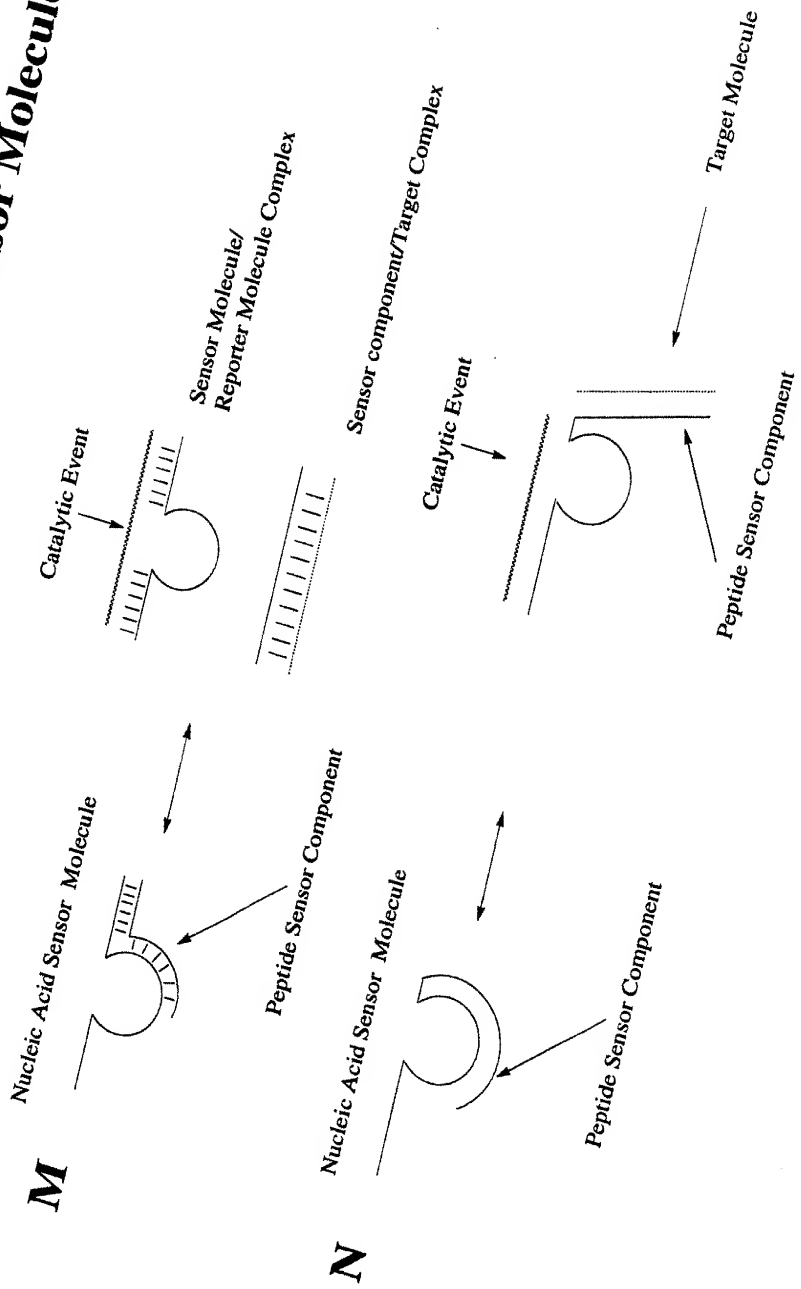


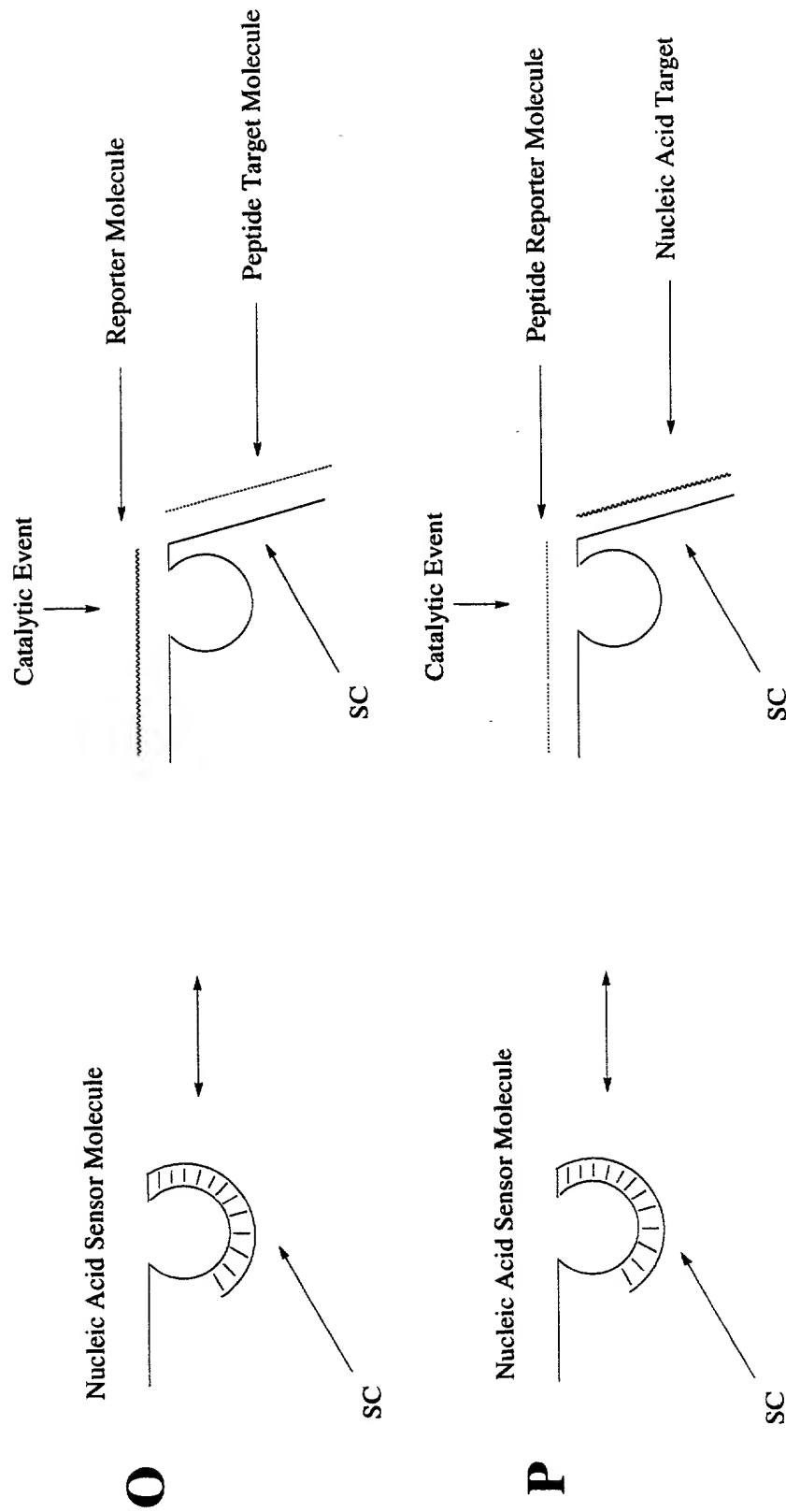
Figure 10: Examples of Nucleic Acid Sensor Molecules



T02001" 32344060  
**Figure 11: Examples of Nucleic Acid Sensor Molecules**

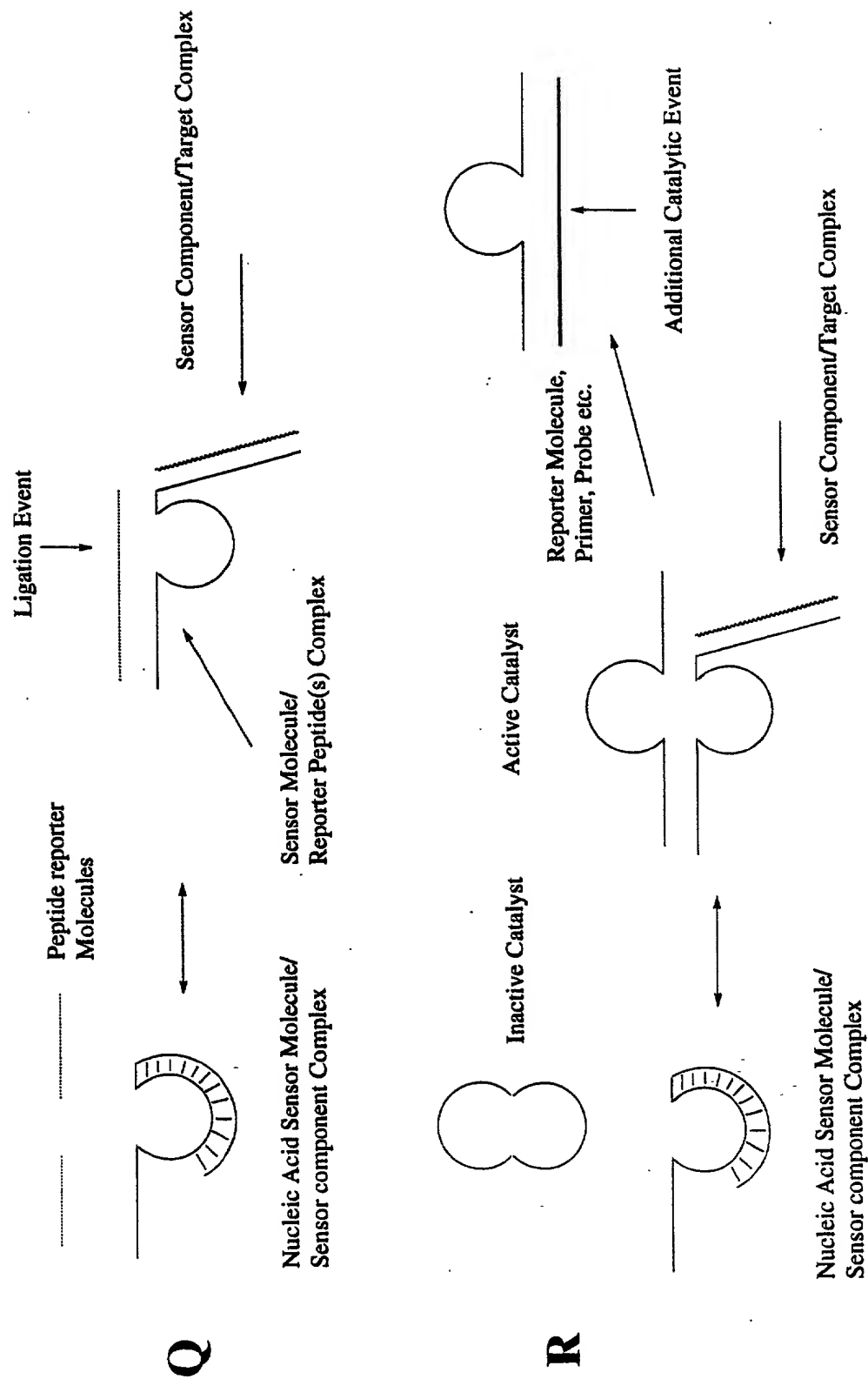


**Figure 12: Examples of Nucleic Acid Sensor Molecules**

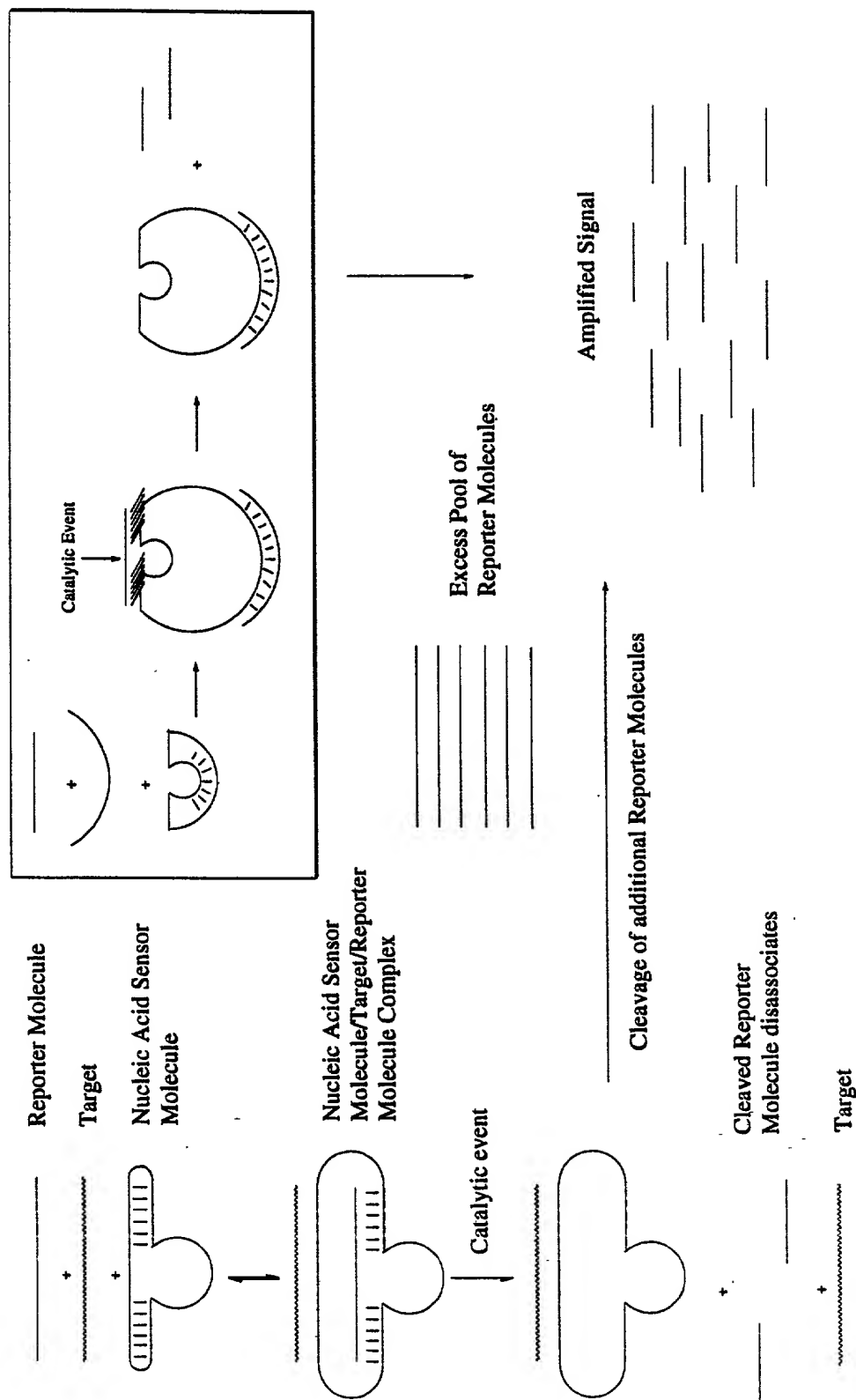


SC = Sensor Component

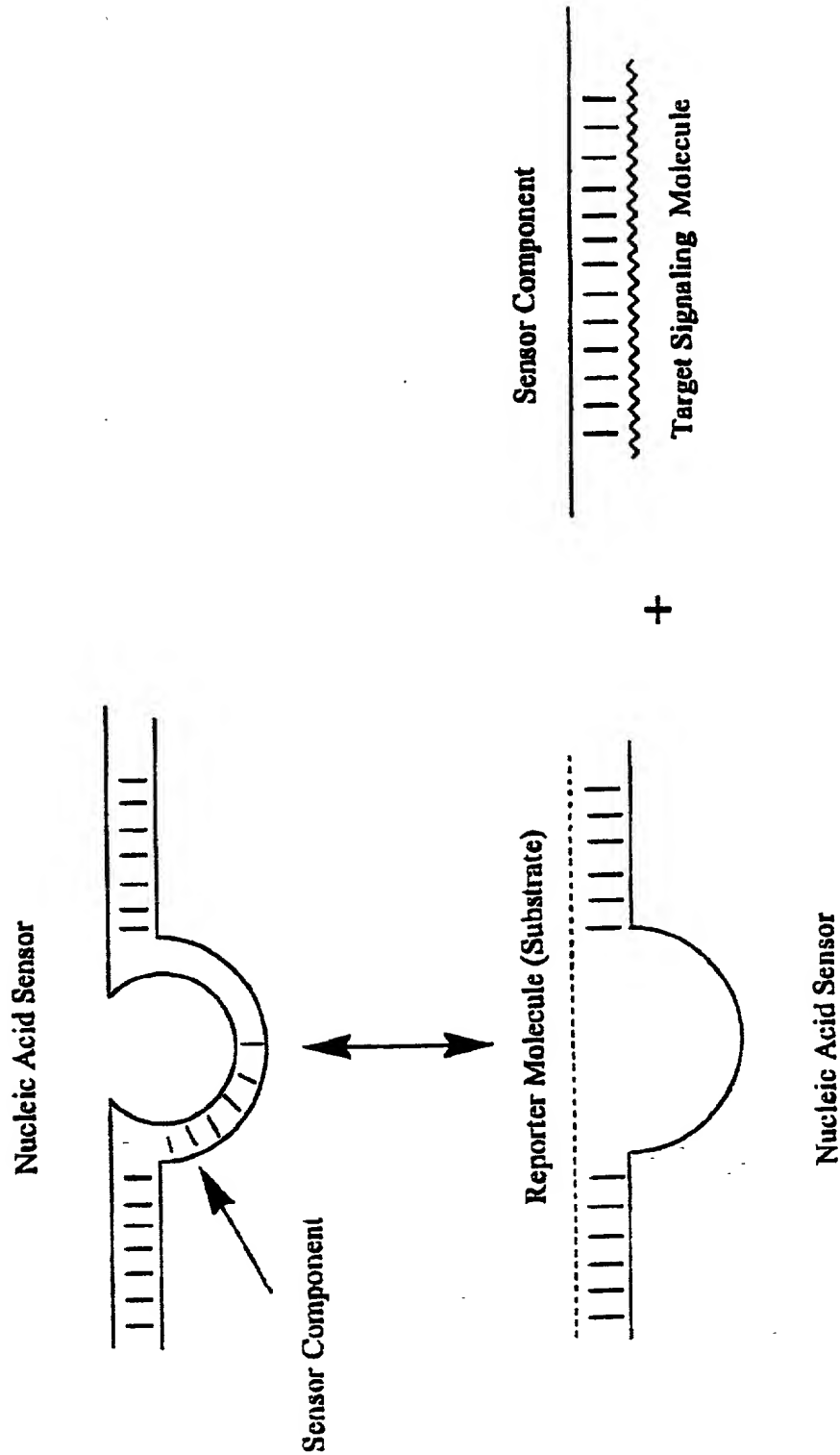
**Figure 13: Examples of Nucleic Acid Sensor Molecules**



**Figure 14: Inherent Amplification of Signal**



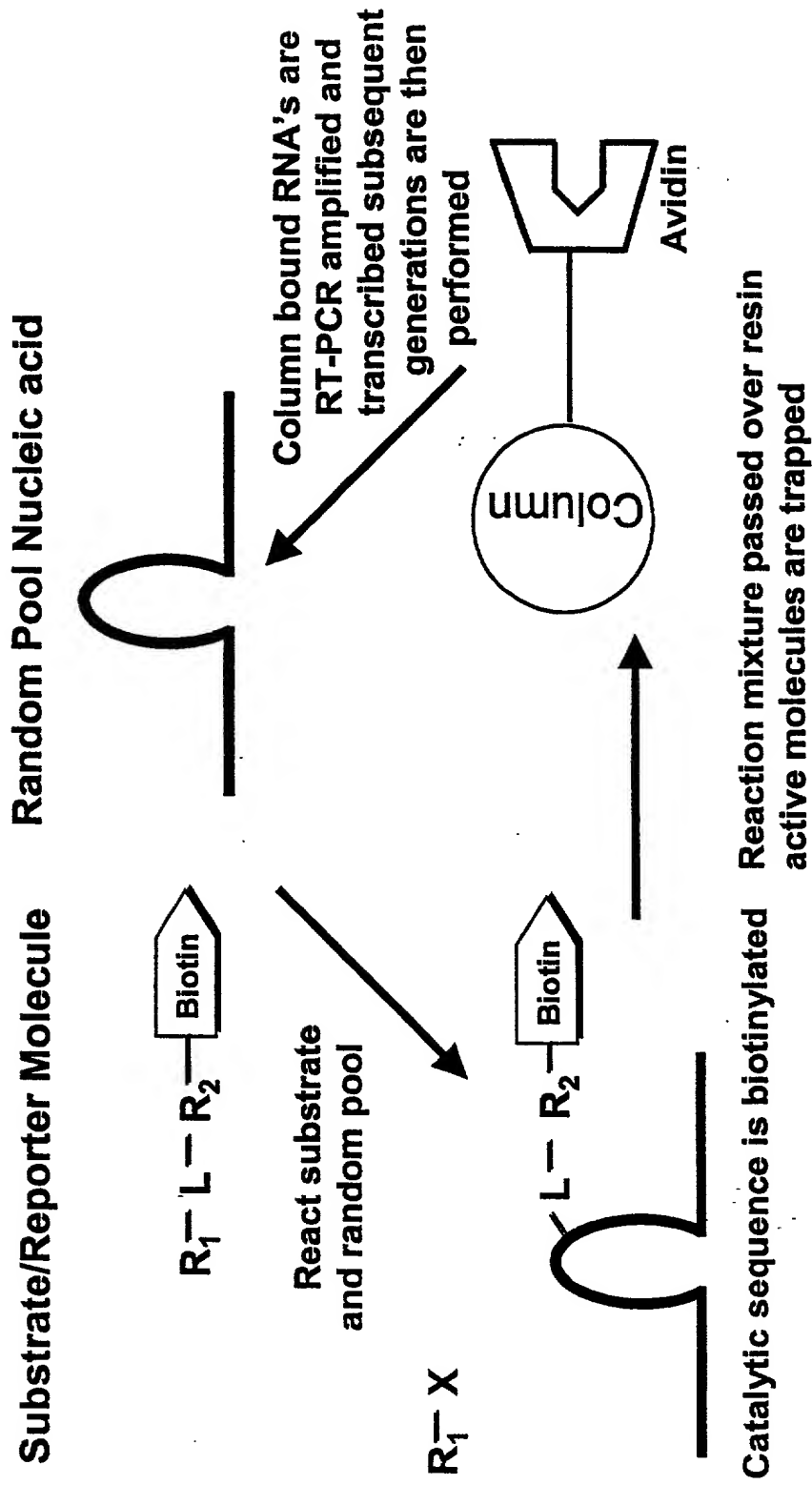
**Figure 15: Example of Diagnostic System**







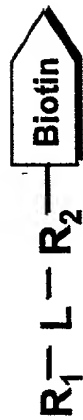
**Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme**



## Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent

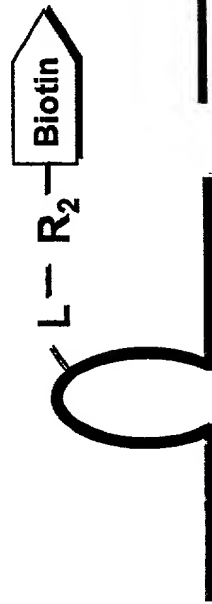
Substrate/Reporter Molecule + Random Pool Nucleic acid

- Ligand (first round)
- + Ligand (second round)



React substrate  
and random pool

$R_1 - X$



### Scheme I

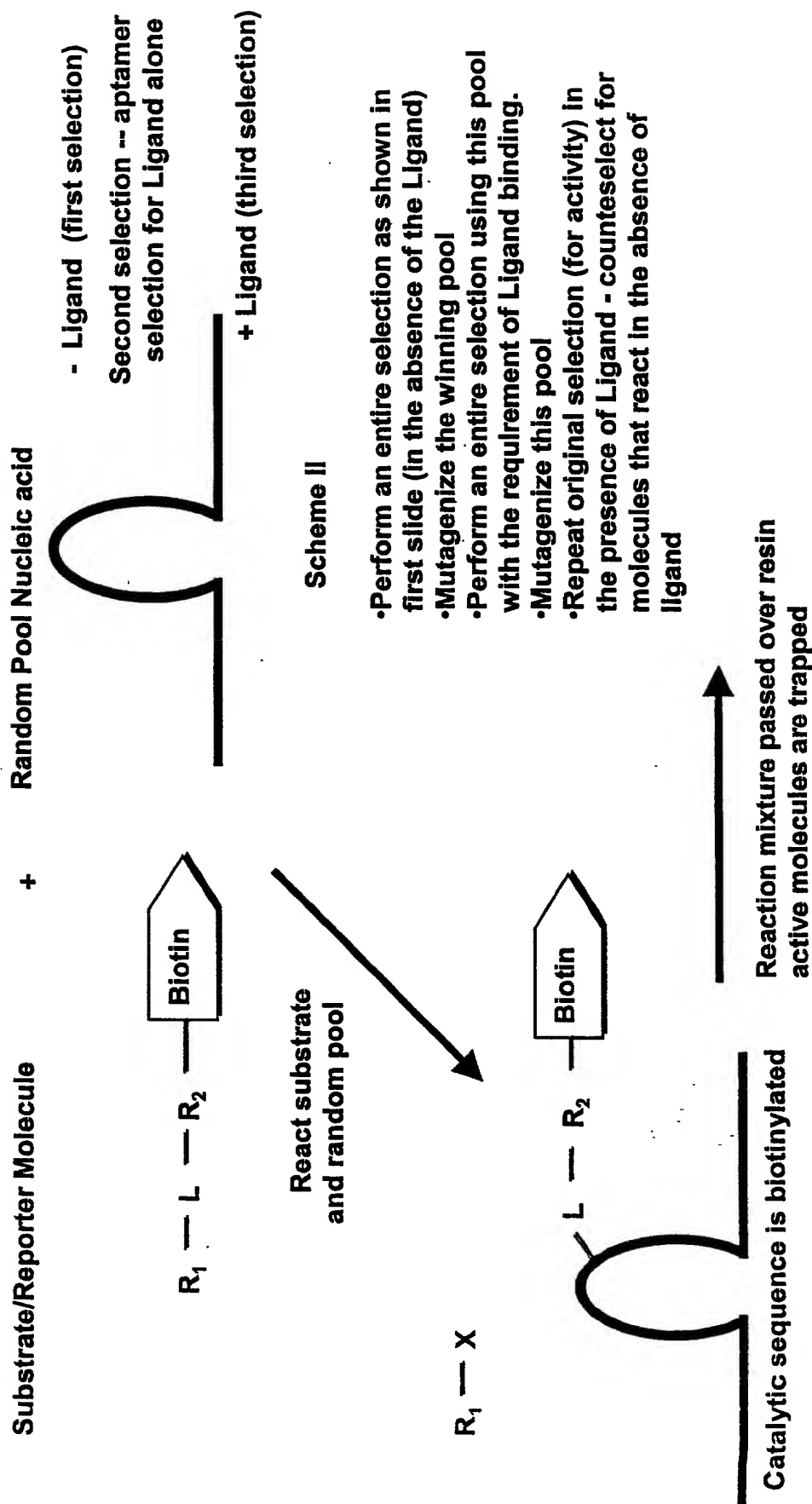
Perform this reaction (in the absence of the Ligand) and disregard the molecules that bind to the avidin resin.

Collect all RNA's that flow through the avidin resin and repeat the reaction in the presence of the Ligand. Collect and RT-PCR amplify and transcribe these molecules for subsequent rounds.

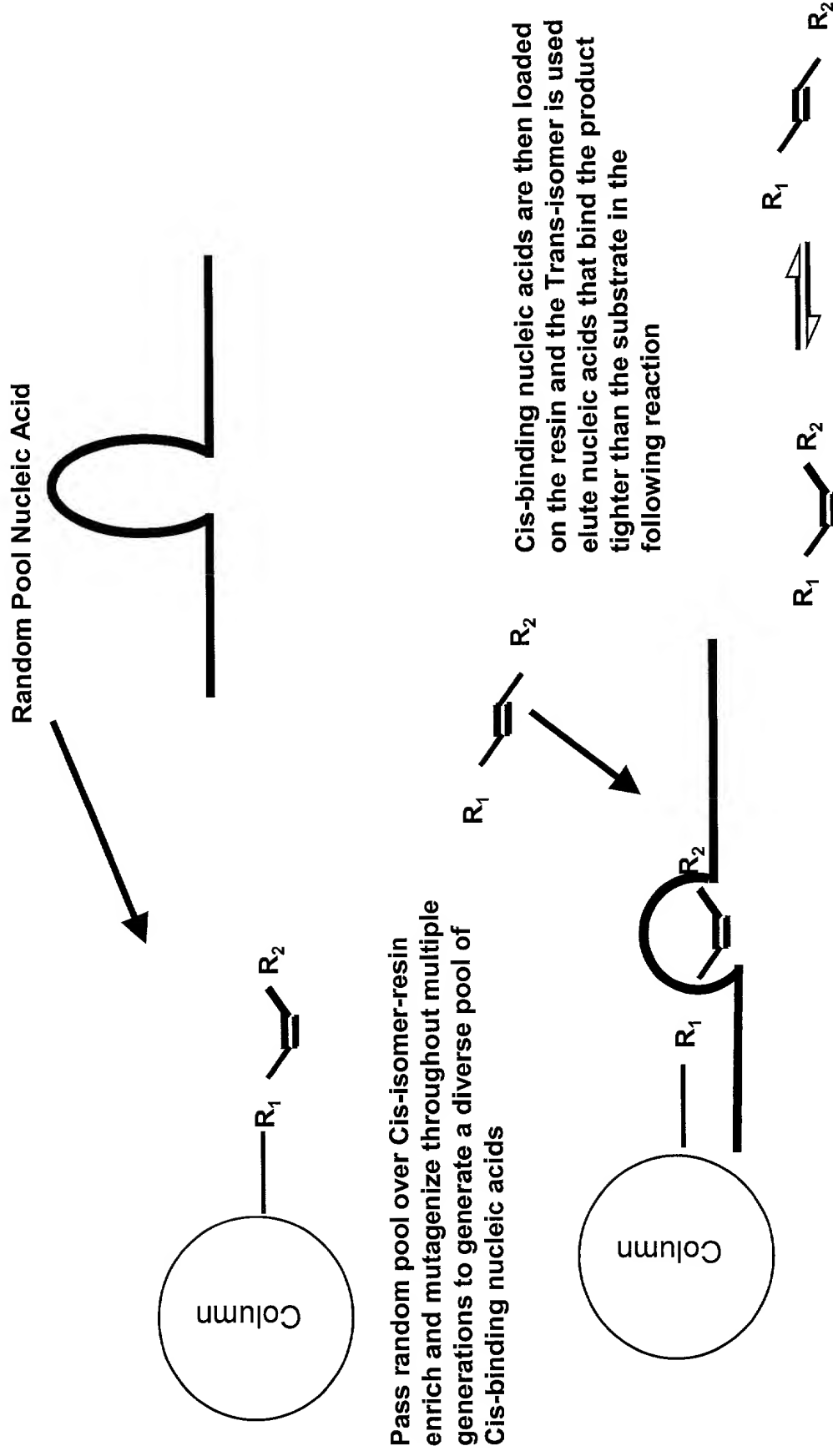
Catalytic sequence is biotinylated

Reaction mixture passed over resin  
active molecules are trapped

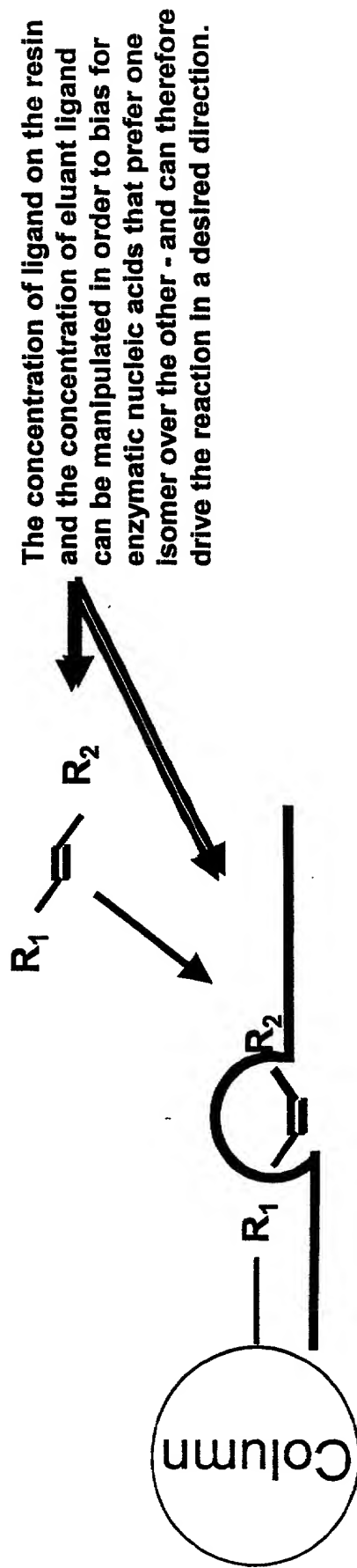
# Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules - Ligand dependent



**Figure 18a: Isomerase Nucleic Acid Sensor Molecule – Selection Scheme**



**Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme**

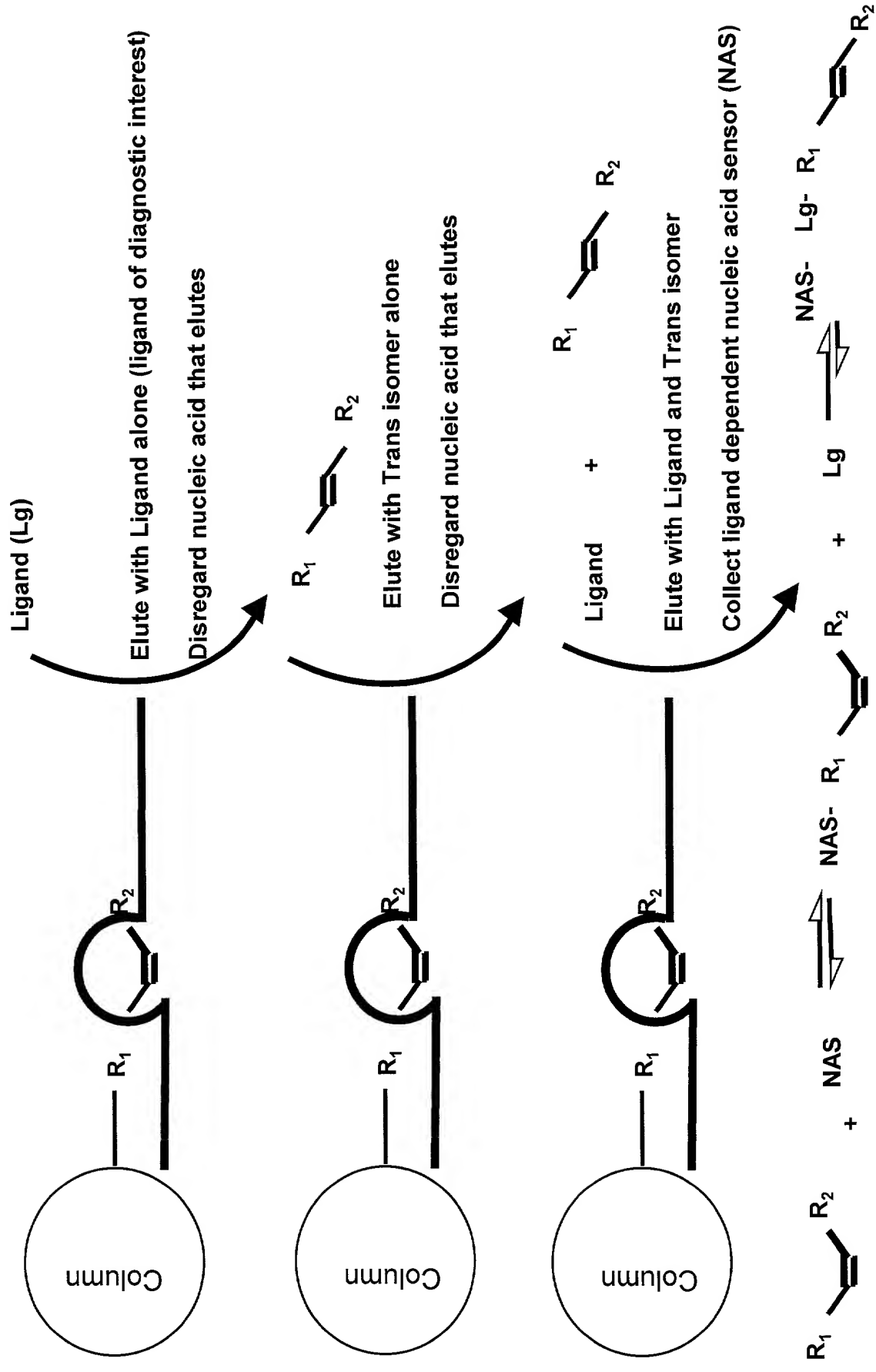


E.g. Selection for Cis-Isomer at 100  $\mu\text{M}$  - yield  $\text{cis}K_d = 100 \mu\text{M}$   
 Elute with Trans-isomer at 0.1  $\mu\text{M}$  - yield  $\text{trans}K_d = 0.1 \mu\text{M}$

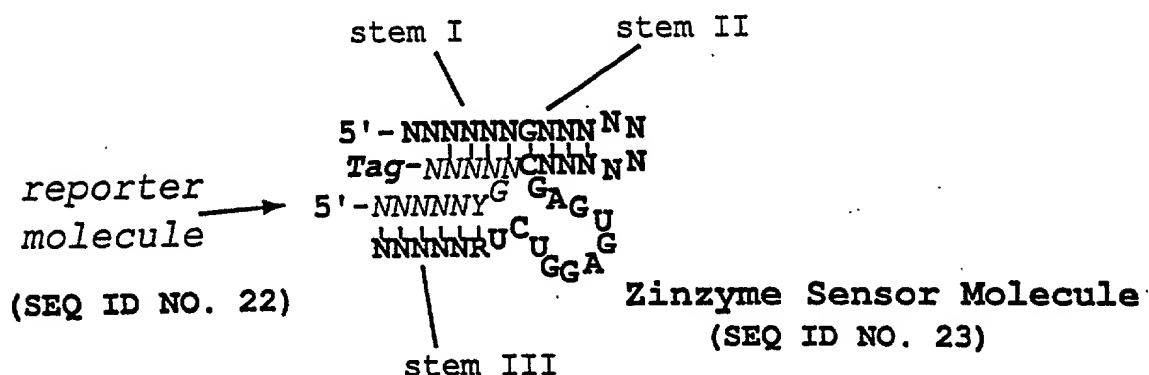
Isolate catalysts for the reaction below



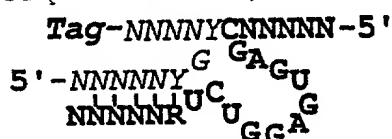
**Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent**



# Zinzyme Sensor Molecule for detection of Nucleic Acid



Inactive Zinzyme sensor/  
reporter molecule complex  
(SEQ ID NO. 22)

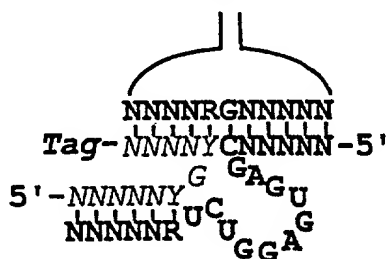


(SEQ ID NO. 24)

Target Signaling  
Molecule



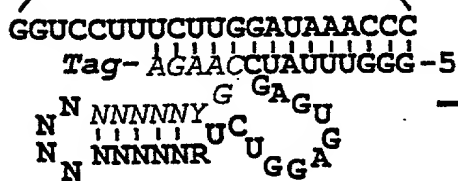
Target Signaling  
Molecule  
(SEQ ID NO. 25)



Active Zinzyme sensor/  
reporter molecule complex

Stem-loop III of HCV (SEQ ID NO. 26)  
(SEQ ID NO. 22)

Active HCV Zinzyme sensor/  
reporter molecule complex  
(SEQ ID NO. 27)

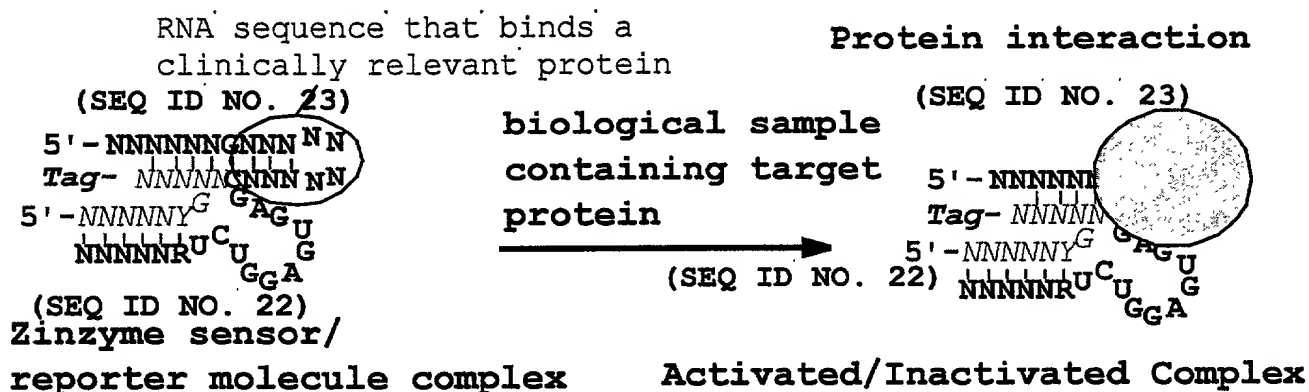
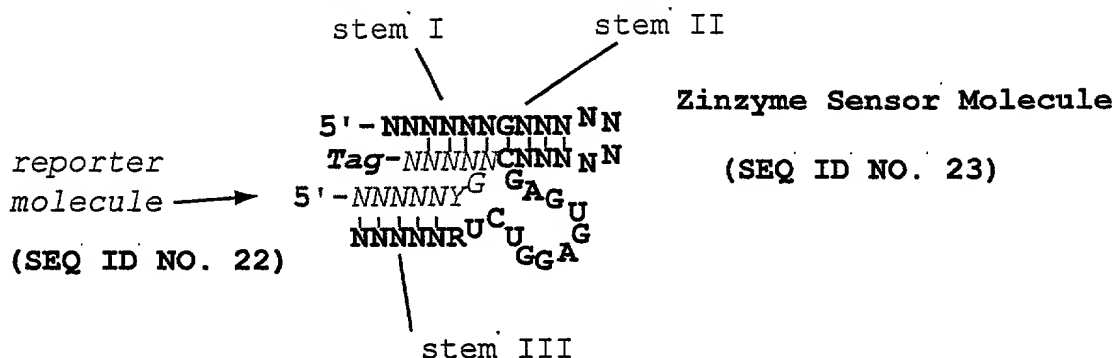


catalysis results in  
release of Tag-AGAAC  
for detection

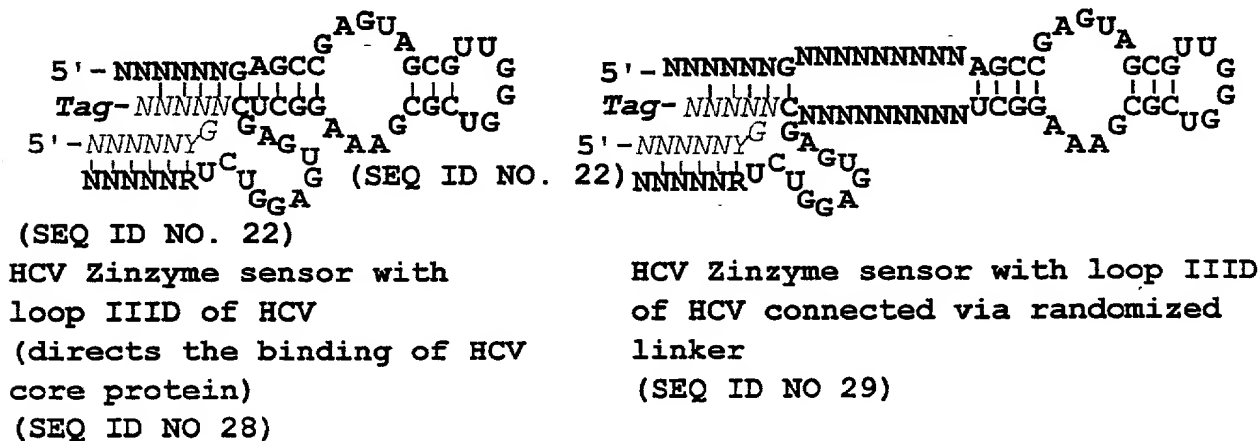
Zinzyme sensor can be attached to solid support/surface,  
for example at the 5'-end

FIG. 19

# Figure 20: Zinzyme Sensor Molecule for detection of Protein

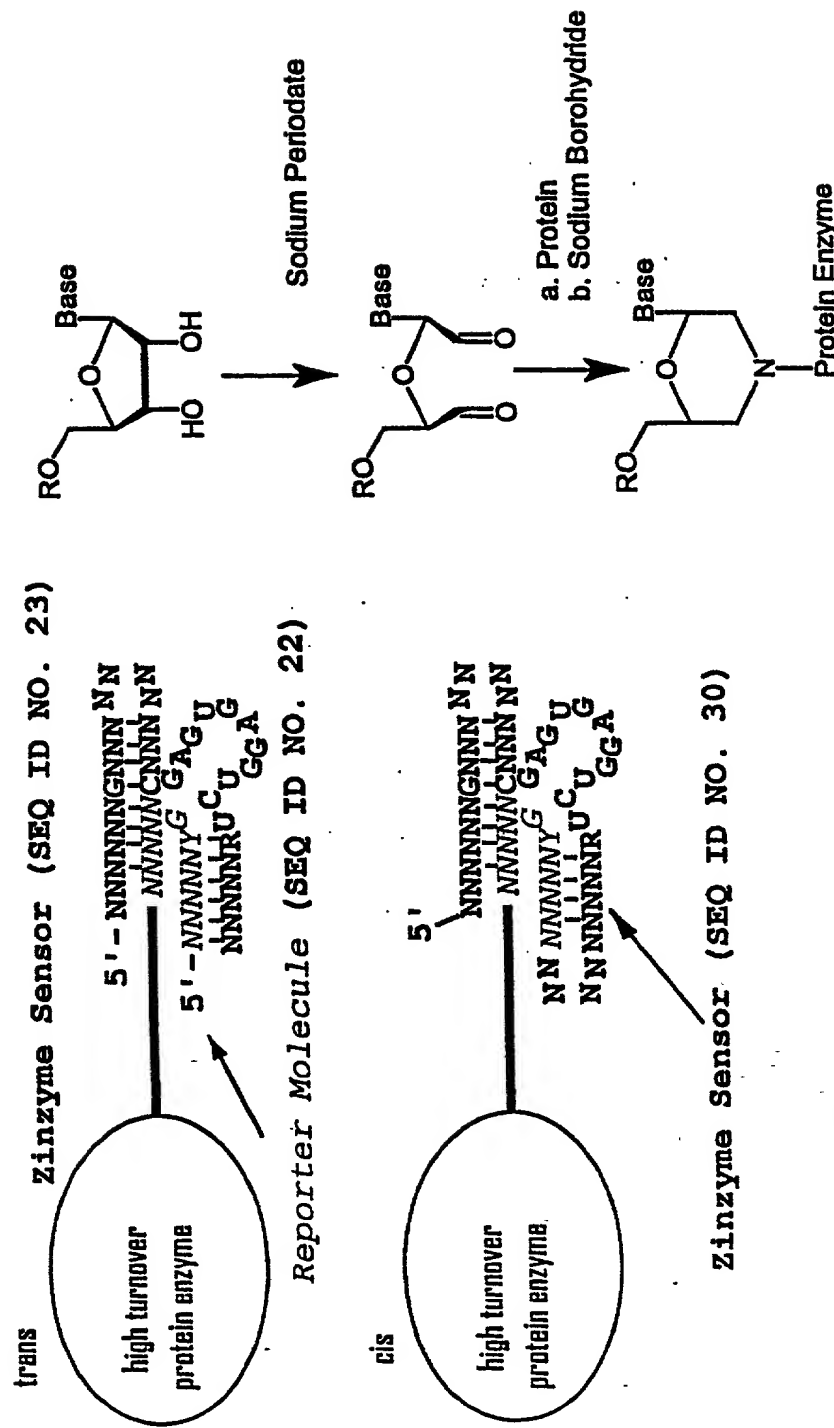


## Sensor/reporter complex for detection of HCV core protein





# *Zinzyme Sensor Molecule with protein enzyme reporter*



R is oligonucleotide.

Protein can be attached via amino linker.

Alternately, R is phosphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

**FIG. 21**

## Amplification of signal via use of protein enzyme conjugate

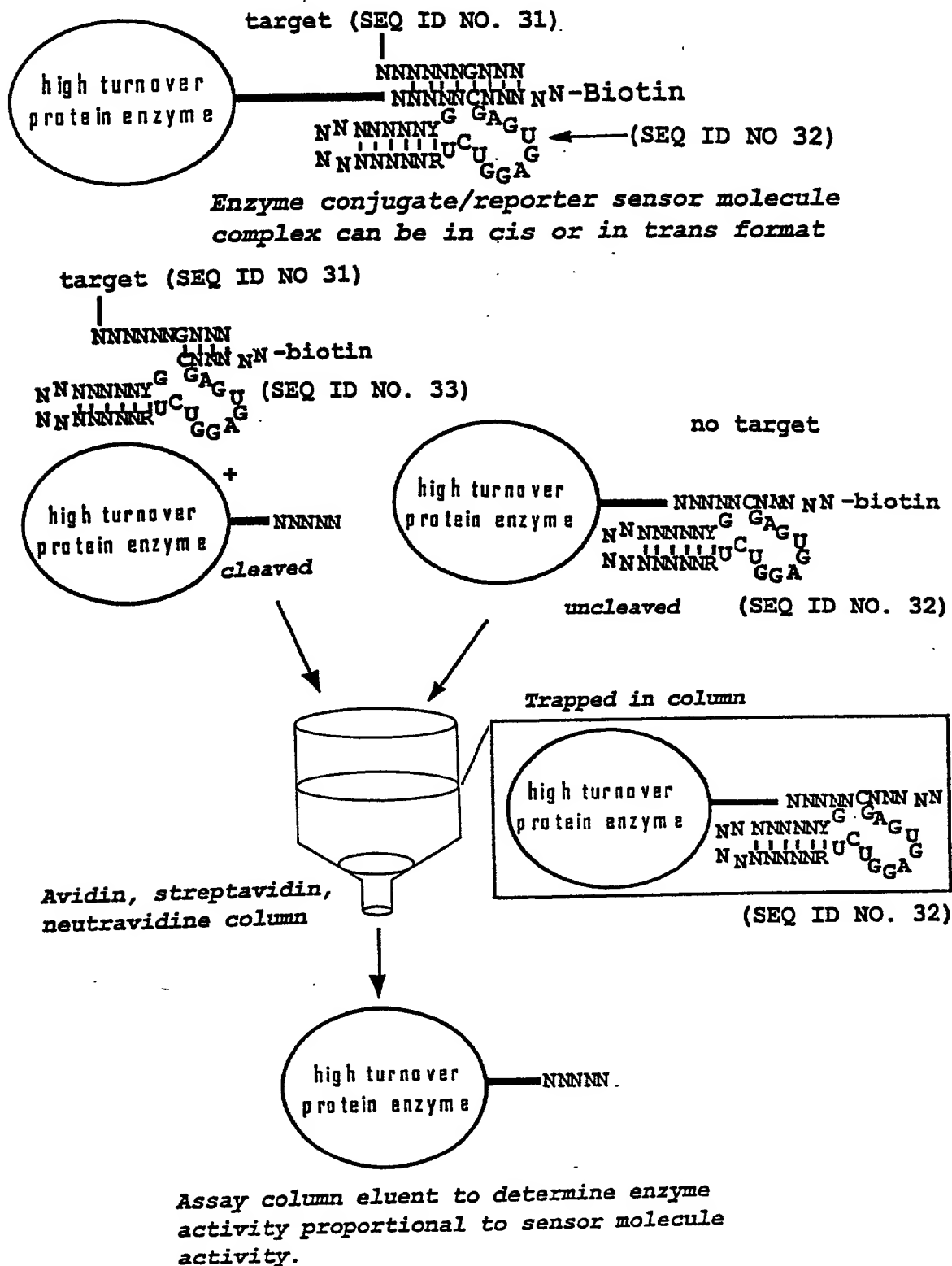
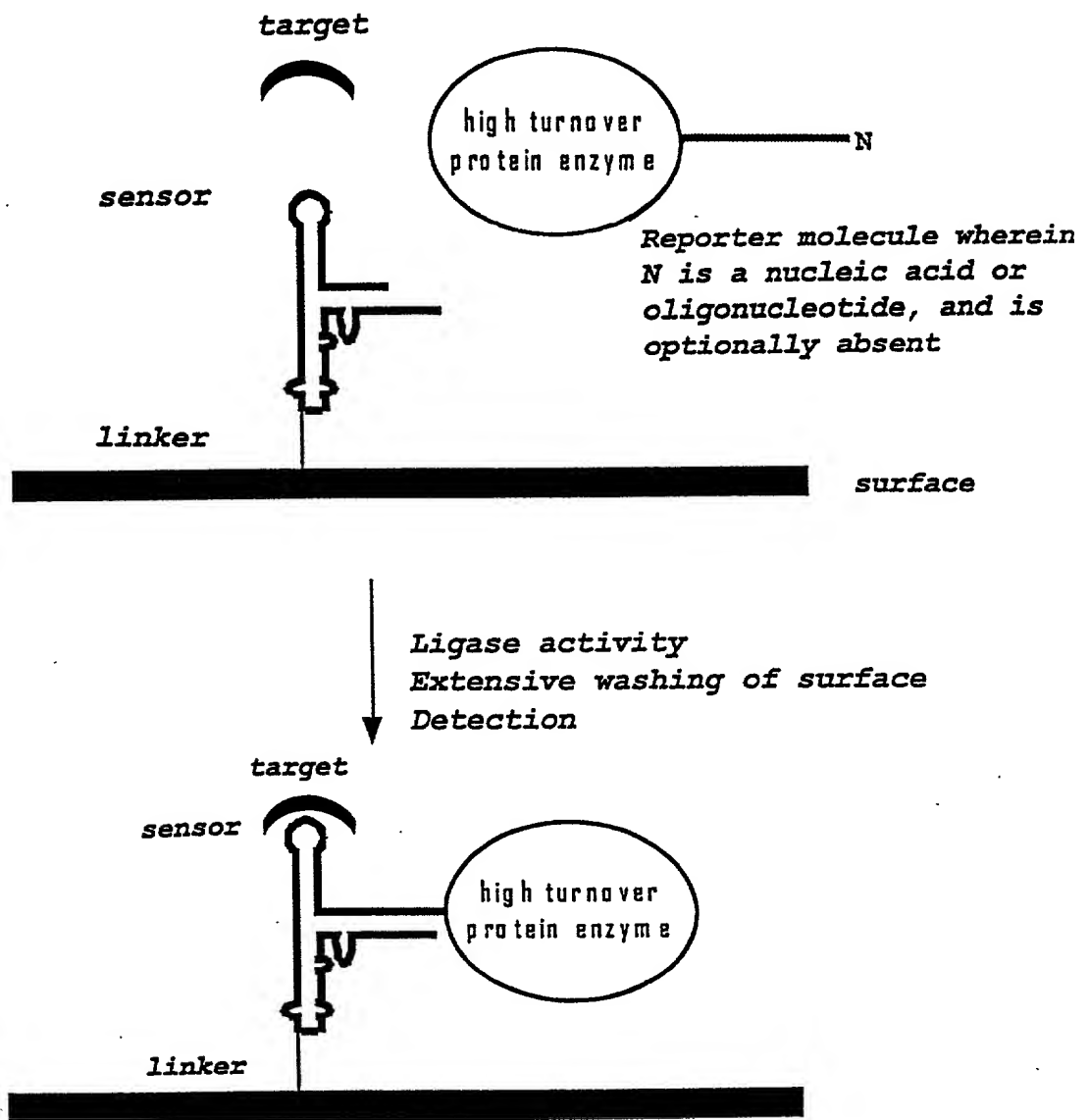


FIG. 22

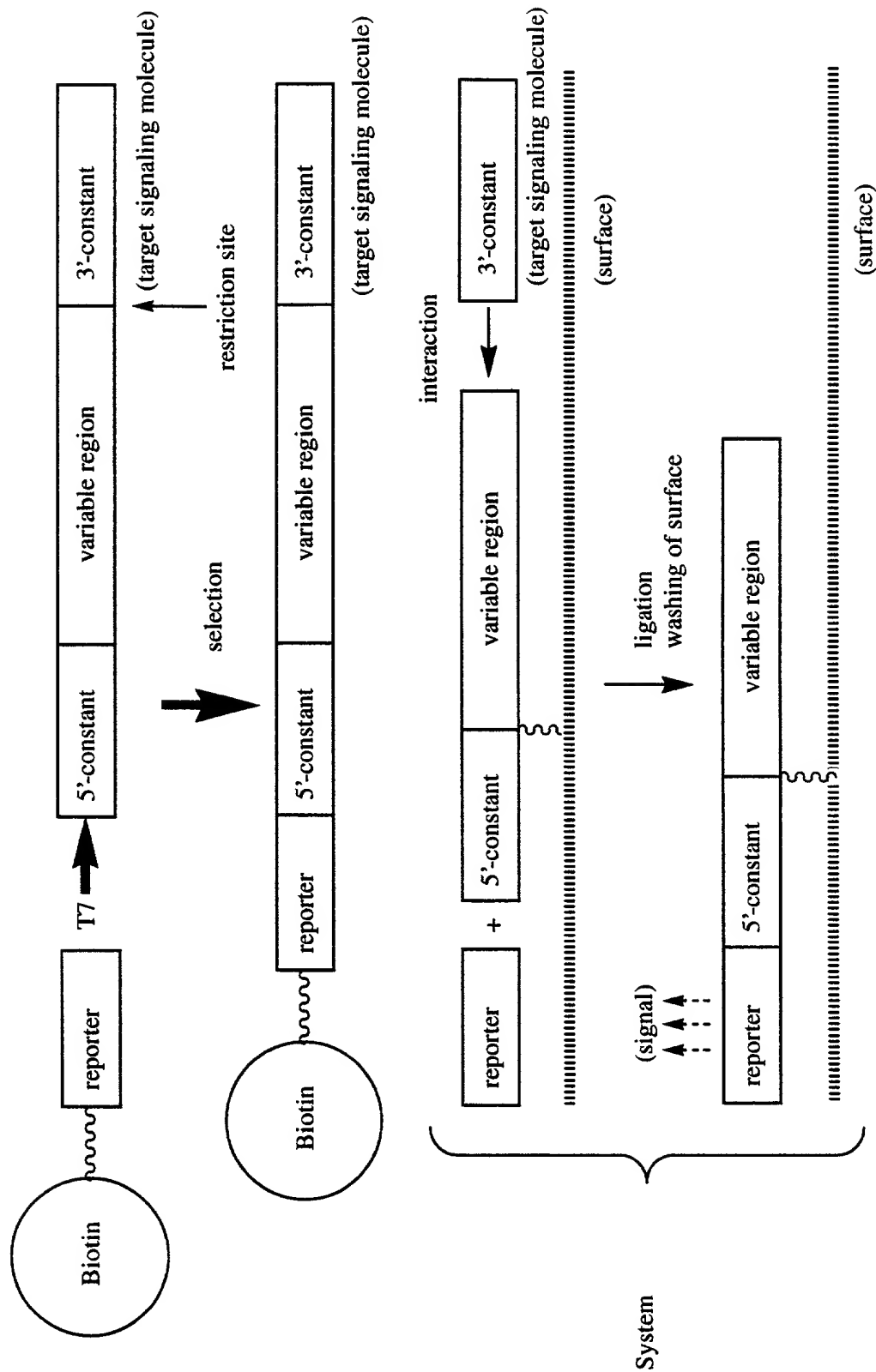
# *Ligase Sensor Molecule with enzymatic reporter*



Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

**Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity**



**Figure 25: Nucleic Acid Sensor Molecule-Based Electric Circuit**

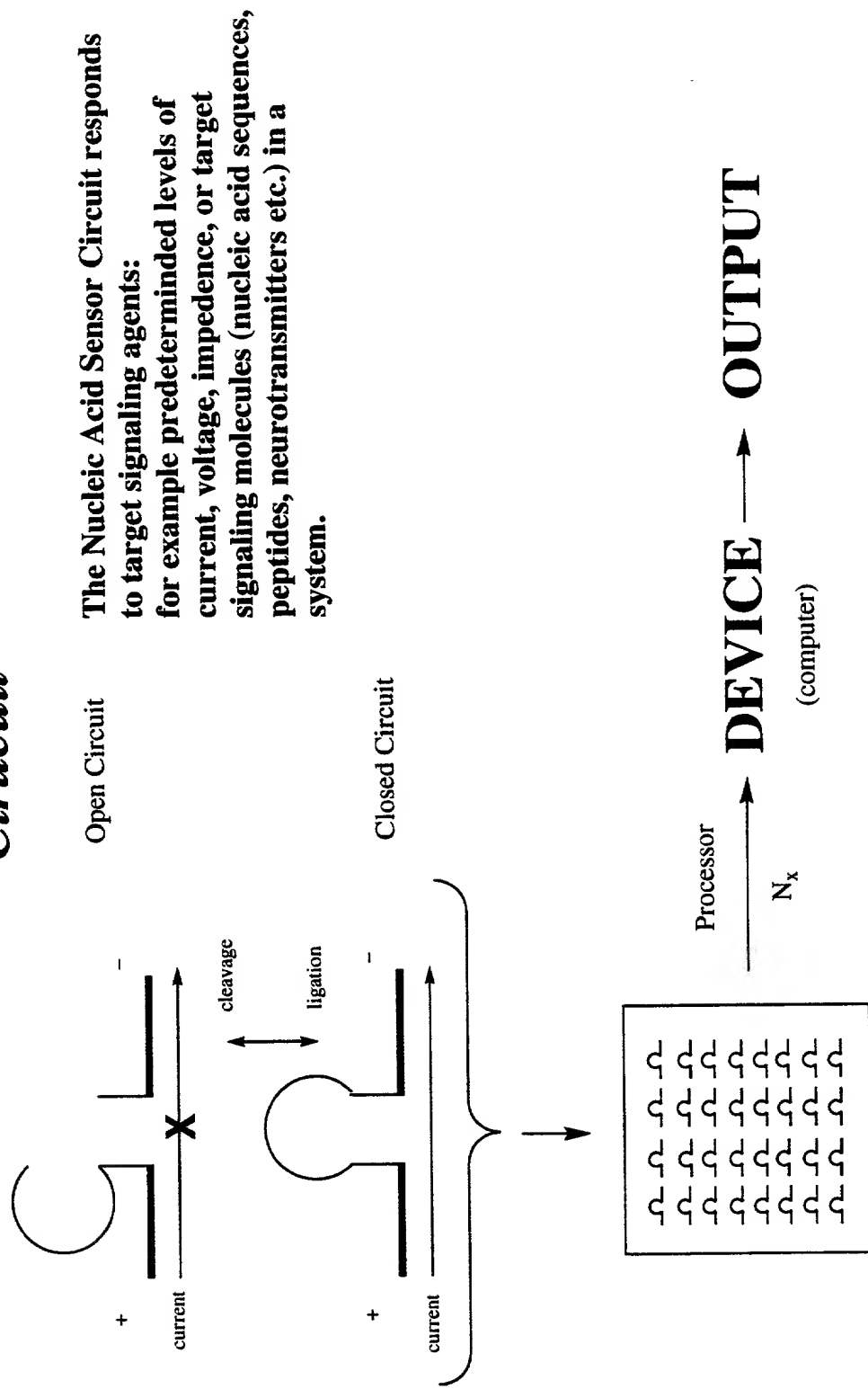
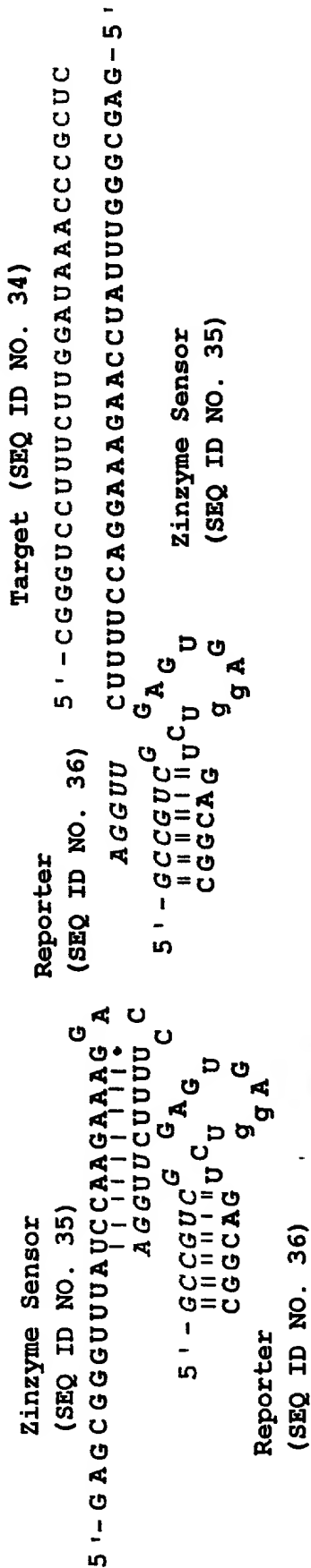
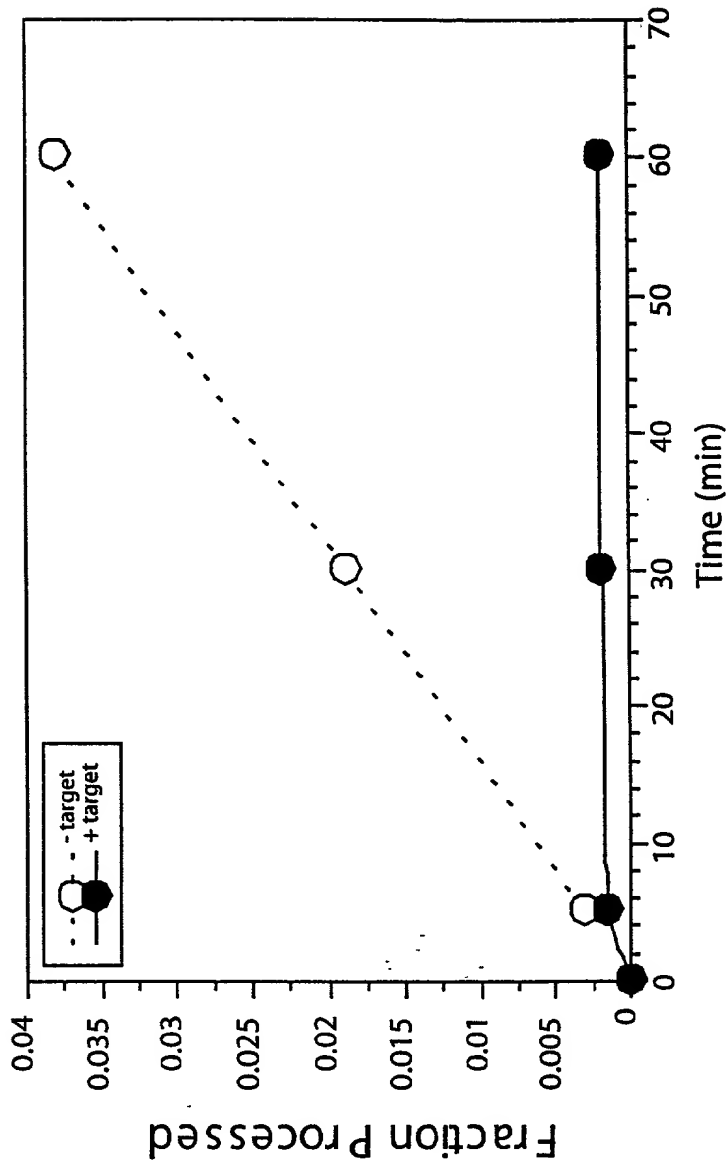


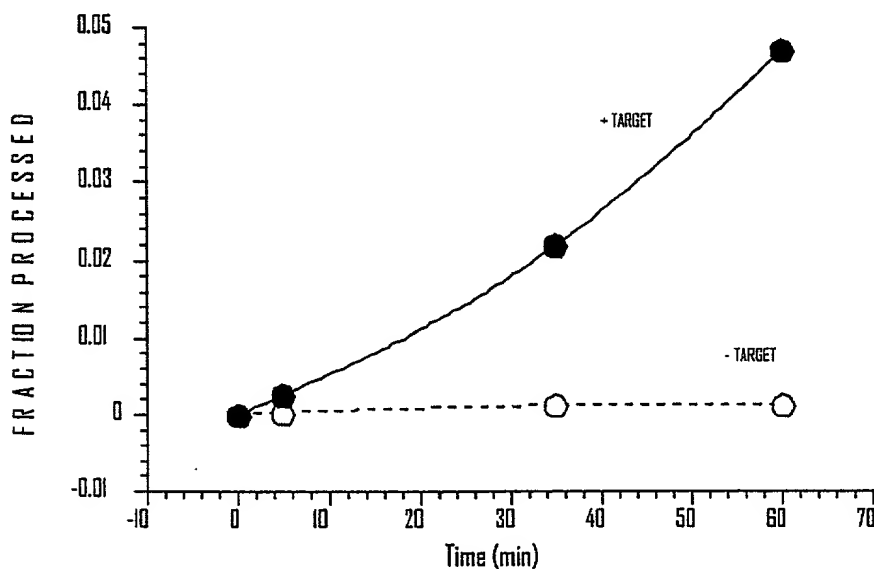
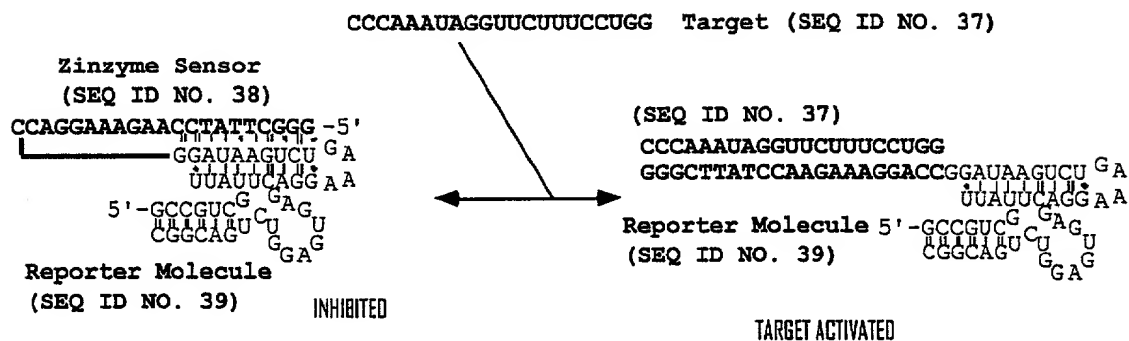
Figure 26: Target Inactivation of Zinzyme Sensor Molecule



ACTIVE  $\longleftrightarrow$  TARGET INACTIVATED



**Figure 27: Target Activation of Zinzyme Sensor Molecule**



09877526-100201

Figure 28: Erk modulated Nucleic Acid Sensor Molecule

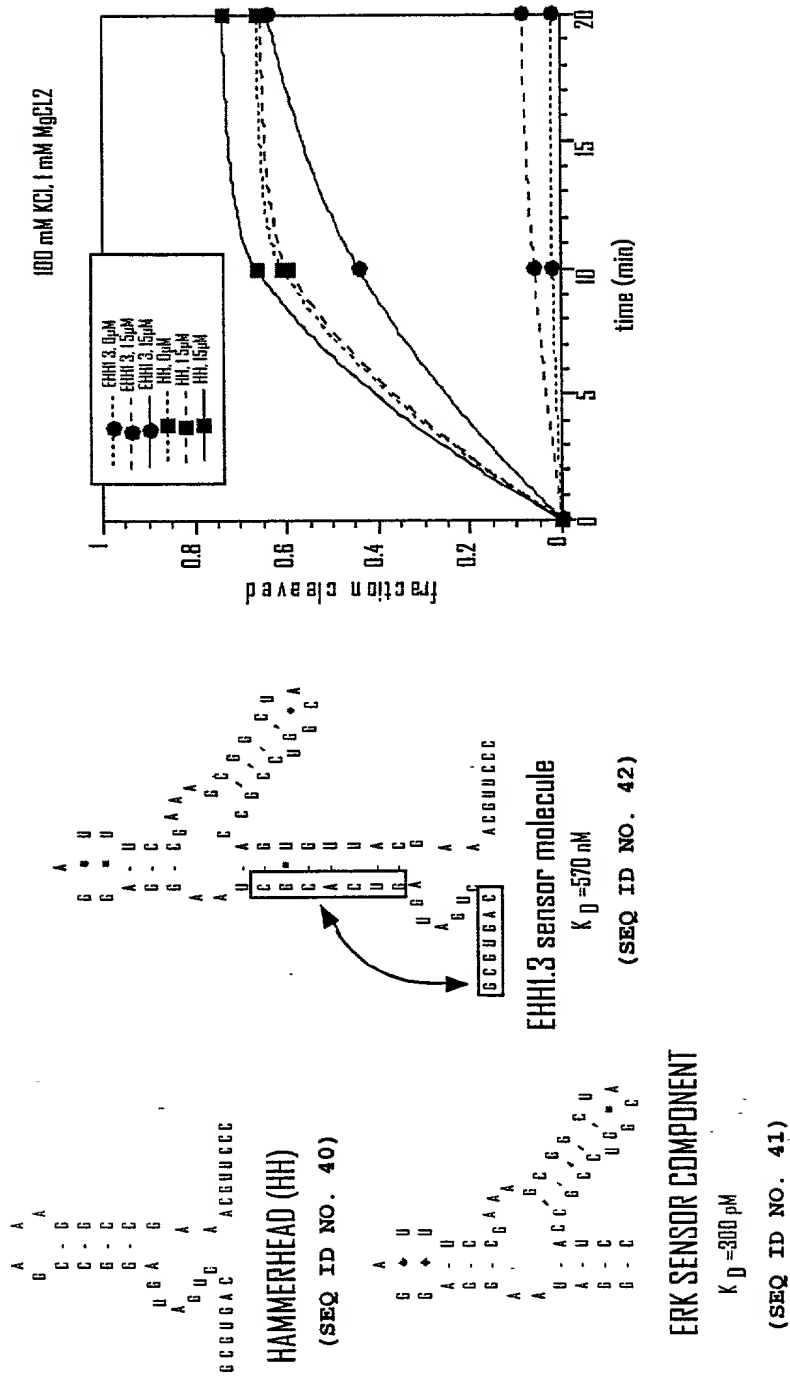






Figure 30

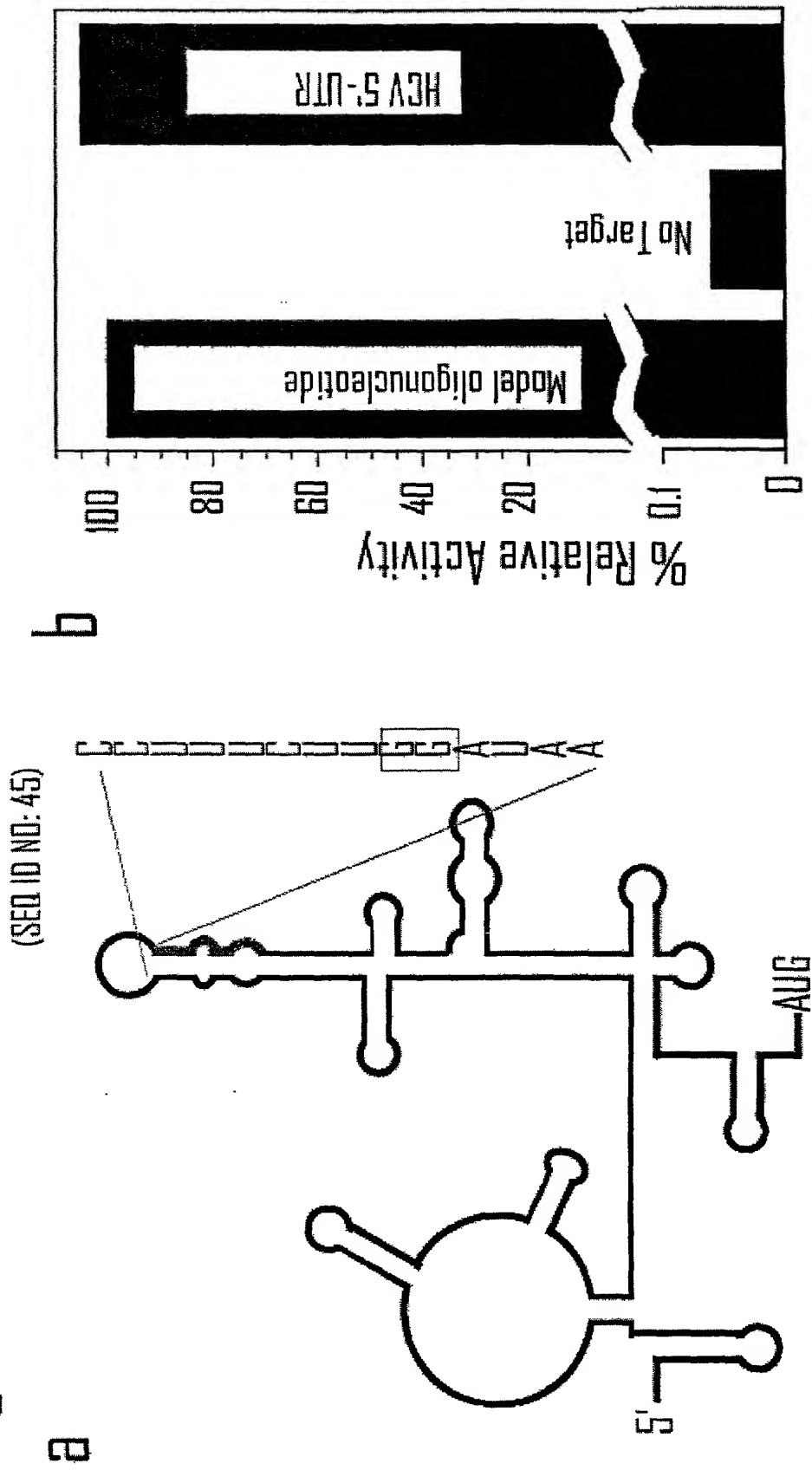


Figure 31

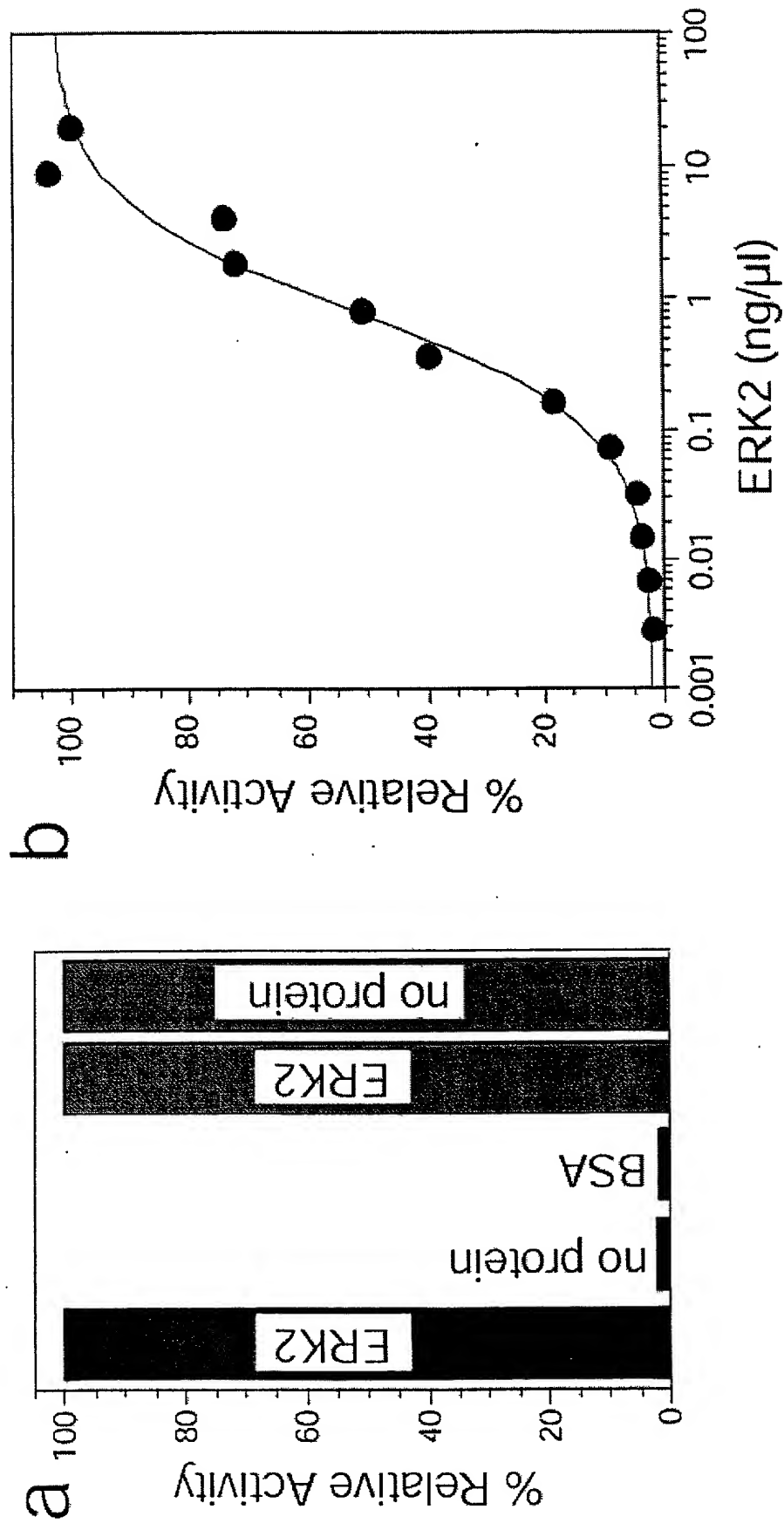
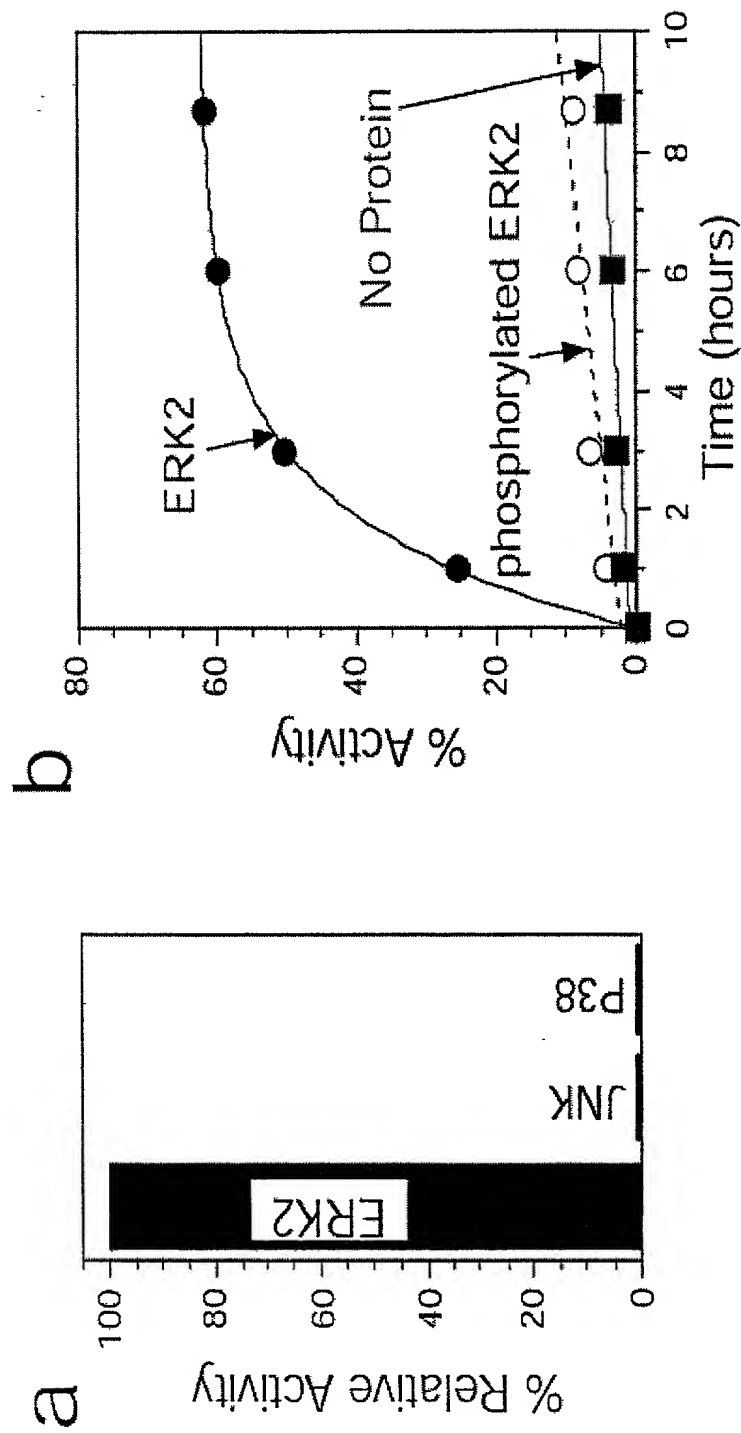


Figure 32



**Figure 33: Halfzyme Ligase**

